CEREAL CHEMISTRY

VOL. XV SEPTEMBER, 1938 No. 5

THE IMPORTANCE OF RESEARCH IN THE CEREAL INDUSTRY

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(Read at the Annual Meeting, May 1938)

The highly efficient cereal industry that we know today didn't exist 50 years ago. In those days the growing, harvesting, and milling of cereals was largely a local matter. Certain farms were recognized as producing better cereals than others. Wheat of one year was compared with wheat of another year, but standards were not set with any degree of certainty, and grades were very indefinite unless the product happened to be damaged. And in that case the grades were about like those of the West Virginia egg buyer who recognized only three grades of eggs—good, cracked, and rotten.

But all this has been changed. The establishment of large central milling plants which command supplies from a wide territory, the demand for uniformity in marketable products, increasing competition in all lines, and the desire for more accurate knowledge by the consuming public have revolutionized the cereal as well as almost all other industries.

In the days before the advent of the Food and Drug Act the story is that many industries attempted to hold customers by other than ethical means such as adding opium or Indian hemp to cigarettes, cocaine to patent medicines, or codein to children's remedies. The cereal industry never stooped to such methods but turned instead to research to solve its increasing host of problems.

The cereal industry today is so broad and its products so numerous that it will be necessary for me to confine my remarks to one or two phases if I am to leave room for others on this program. And I expect to do that. So in passing, I merely wish to state that cereal products are used for both human and animal consumption and to an increasing extent by industry which accounts for the consumption of a good many million bushels of grain each year. My remarks today will be devoted principally to that portion of the cereals which find their way eventually to the table in the form of bread.

The family that sits down to the dinner table of today has a much better opportunity to partake of a balanced diet than the family of 50 years ago. Research and transportation are responsible for this improvement, and the cereal chemist is entitled to a great deal of credit for the part he has played in bringing about the change.

The American cereal chemist occupies a position of great economic importance in the lives of our people. The feeding of 130 million people three times a day is quite an undertaking. Merely furnishing the bread for the people of this country is a large item in itself. The commercial bread bill, which is only about half of the total bread bill, amounted to about $600,000,000 in 1932, but by last year it had climbed to more than $700,000,000. The materials, such as flour, milk, sugar, yeast, shortening, malt, and so on, that were used in baking last year's bread amounted to about $100,000,000, with a total consumption of more than 33 million barrels of white flour for commercial bread baking alone. When we include the baking done by housewives, institutions, corporations, etc. we need about double that amount of flour to meet the annual bread baking requirements of our population. Since it requires about 4½ bushels of wheat to produce a 196-pound barrel of white flour you can readily understand that it requires approximately 300 million bushels of wheat to meet the annual bread requirements of the country, and about 450 million bushels for our domestic food requirements. Of the cereals grown for human consumption about 95% is from wheat. Our annual milling of cereal grains in order of importance is about as follows:

White flour	95	million barrels
Semolina	3	million barrels
Prepared flour	2½	million barrels
Graham flour	1½	million barrels
Corn meal	7	million barrels
Rye flour	1½	million barrels
Buckwheat flour	25	million pounds
Other flour	14	million pounds
Bran and middlings	4	million tons
Feed, screenings	1½	million tons

This at a glance gives you an idea of the importance of wheat flour in bread and bread making and explains why I am dwelling upon wheat more than the other cereals and upon white flour more than the others mentioned. It is estimated that about 30% of the value of the foods processed in the United States each year is composed, in part or in whole, of cereals. This does not take into account the 130 million bushels of cereals used in brewing and distilling, or that used for other purposes.

Wheat is recognized as nutritionally important in the diet. Wheat flour accounts for over a third of the calories and about one-fourth of

the protein in the average American diet and of a much larger proportion in the diet of families of low income. The cereals, especially in the whole form, are important sources of some of our most valuable minerals and vitamins. All in all, the cereals are important items in balancing the diets and improving the health of the people, and it is the job of the cereal chemists to search out the truths which will be of benefit to this great industry and to the public at large. The average consumer knows very little about the mineral and vitamin content of the food he eats and the cereal chemist is in a position to furnish some of this information, especially with reference to cereal products.

Now with this rather lengthy introduction let's get a little closer to our subject by taking a look at some of the problems that confront the cereal chemist. As you will note I am taking the attitude of the negro preacher who said he always chose a text, strayed away from it, gave two illustrations, and if time permitted got back to his text again before he closed his service. To begin with, we'll all admit that the main object of research in the cereal industry is to produce more wholesome foods—foods that are better fitted and adapted to our body needs, and foods that will help maintain health and vigor, and, if possible, prolong life. It has been said that one-third of our people are ill fed. If that is true, and there seems to be little doubt about it, the cereal chemist can well afford to point out the foods in his field that furnish cheap, dependable sources of such important things as minerals and vitamins. I have heard it said that the average American skirts dangerously close to the minimum of sixty-eight hundredths of a gram of calcium that is needed by the body each day. If that is the case something might be said about specific foods that are good sources of calcium—for example, the milk that is used in bread making. The dissemination of such information would not only help the public, but it would help the cereal industry by increasing the demand for bakery products that are high in this important element. What I have just said can hardly be considered in the class of research but rather the dissemination of information which is the result of research.

Another problem that confronts the cereal industry and one that the cereal chemist is interested in is the declining consumption of wheat flour. The decline in flour consumption since the beginning of the century has been over 25%. If the bread consumption of today were equal to what it was 30 years ago farmers would need to send 175 million more bushels of wheat to the mills than they are sending today. The annual per capita consumption today is around 170 pounds of wheat flour, 30 pounds of corn, and 6 pounds of rice. And that, as I have just pointed out, is considerably less than it used to be. It has been said that we eat too much cereal foods for our good, but I have

never heard it said that bread could not be made to fit into the scheme of a well-balanced diet. It is true that modern transportation has provided the American family with more fresh vegetables than we had 50 years ago. It is also true that we are consuming more milk and eggs than we used to. All of these foods are good and are most essential for a well-balanced diet. But when it is all summed up, we find that good wholesome bread is still important in the well-balanced American diet, and if the consumption of flour has fallen off in the last 25 years there are probably reasons, and I think it would do no harm for the cereal chemists to point out some of these reasons.

Let us pursue the subject a bit further. As you are well aware, the increased use of shortening, skim-milk powder, sugar, malt, salt, and so on have made it possible to use more water per barrel of flour. For example, 30 years ago it required 72 pounds of flour to make 100 pounds of bread. Today, because of the increased use of adjuncts, the same amount of bread can be made from only 65 pounds of flour. This one factor alone accounts for a loss of 2 million barrels of flour a year.

Then there is the question of indifference among some bakers. I doubt if this amounts to a great deal because most bakers want to turn out the best product they can. At least that should be their goal, and the bakers who strive to turn out appetizing bread of the highest quality are living testimonials that it pays to produce bread that the public likes. I have heard it said that at least a part of the decline in flour consumption is due to the fact that too much "clear" flour is going into family flour. It seems to be rather common knowledge that private-brand family flours now absorb several million barrels of "clears" each year that used to find foreign outlets. This has probably come about as the result of heavy bleaching.

Research has also revealed that our modern method of harvesting wheat has had some effect upon the quality of the loaf of bread. Years ago wheat was stacked or placed in shocks to await the coming of the community thresher. During this wait, which often amounted to as much as two or three months, partial germination took place through sweating, dew, rainfall, and so on, and this affected not only the quality of the wheat but also the quality of the bread produced from it. Flour made from unduly germinated wheat produced an inferior bread, but on the other hand it is generally admitted that a small amount of enzymic activity, or germination, is essential to the making of good bread. Scientific bakers get around this problem when they use flour made from combine-harvested wheat by adding a small quantity of malt flour to the combine-harvested wheat flour.

It is claimed by some that the tendency to use chemical fertilizers

is slowly robbing the soil of some of its minor elements which not only affect the quality of the gluten for baking purposes but even affect the quality of the crop itself. The results of experiments by McCarrison in India indicate that vegetables grown on soil treated with barnyard manure were superior in food value to vegetables grown on similar soils without manure. A question which may be raised is, if the above is true, what effect if any will continuous cropping, soil erosion, and the continuous use of chemical fertilizers have on the quality of flour made from wheat grown on such soils? It is just possible that this may be one of the contributing factors in the decline in the consumption of wheat flour. I say this because scientists are not agreed as to just what effect this change has upon the nutritional value of plants. Anyway, it is a problem which I think cereal chemists could well look into as they are peculiarly qualified to carry on research in this field.

It might be profitable to give more attention to the value of the offal that is produced in milling wheat. This annual offal amounts to more than 4 million tons, containing over 2 million tons of pure bran and a quarter of a million tons of wheat germ. The average price received for this valuable offal is around $35 a ton, or less than 2 cents a pound. The wheat germ, as you cereal chemists know, is a valuable food product. It is rich in a high-quality protein, rich in minerals, and contains six times as much vitamin B and four times as much vitamin G as the whole-wheat flour. The wheat germ is also an excellent source of vitamin E and a good source of vitamin A. "Germ breads," as they are called, are becoming quite popular in England. Possibly our cereal chemists and bakers could work out a better bread by including some of these valuable minerals and vitamins. We have been attempting for several years in this country to produce a loaf of bread which would combine the attractive qualities of the white loaf and have a better balanced bread so far as vitamins and minerals are concerned. It is generally recognized today that vitamins and minerals play an important role in nutritional diets, and since we know that some of this offal is rich in these properties, it may be possible to broaden our research and investigations in this field. In my opinion it is worth thinking of at any rate.

The staling of bread is another subject that the cereal chemist ought to explore more fully. There has been so much said about stale bread that some people have the idea that bread must be rushed from the baking oven to the consumer within the hour it if is to be consumed while it is fresh. While there may be some merit to this idea from the consumer's viewpoint, it is difficult if not impossible to merchandise bakery goods with such speed under normal conditions, and most of

the bread that is consumed today is from 24 to 40 hours old when it is eaten and is still good bread if it has been properly handled. It has been conservatively estimated that something like 500 million loaves, or about 5%, of the bread that is manufactured in this country is returned to the bakers as stale bread. The loss due to this practice is estimated to amount to between 10 and 40 million dollars a year. This is a plum that is worth going after in a big way. Probably we need both careful research and education of the public to save this loss. Staling is certainly not the only thing that affects the taste and quality of bread. Quality is started long before the loaf reaches the staling age. Quality begins with the variety of wheat, extends to the locality where it is grown, the conditions under which it is grown, the chemical composition of the wheat after it is grown, the gluten quality of the wheat, conditions of storing and milling, the treatment of the flour after the wheat is milled, the formula and method used in baking, and so on. All of these are subjects for research for the cereal chemist.

Rope in bread is sometimes a cause of serious loss to the baker. During warm weather rope in bread is occasionally encountered unless the baker has taken the proper precaution to acidify his dough. It is generally believed that a quick-rising yeast bread is more likely to develop rope than one that has had a long period of fermentation. It might be wise to conduct some investigations to show the chemical changes that take place in dough as the result of long and short fermentation. Such investigations might lead to a better understanding of the nature of some of our important bread diseases. It might enable us to prevent them.

The storage of bread is as yet of no great importance in this country, but it might become important in case of war or an emergency. The results of investigations thus far in this field indicate that when fresh bread is frozen quickly it may remain fresh for weeks. On thawing, such bread may show a compressibility almost equal to that of fresh baked bread. What are the effects of sub-zero temperature storage? We have found that in other fields quick freezing and sub-zero storage maintain quality for long periods of time.

With the Ever Normal Granary idea before us the effect of long storage of wheat on the bread produced from this flour should not be overlooked. Some research has already been done in this field but we may need much more information in the near future.

Just a few more suggestions as to the problems which may be worthy of attention.

The addition to wheat flour of varying amounts of flour prepared from legumes that had been germinated for different lengths of time

might yield interesting results both as to quality of loaf and the effect upon staling.

A comprehensive study with different grades of flour regarding the action of the numerous flour improvers (chemical, physical, and biological), yeast foods, bleaching agents that have only a bleaching action, and those that also act as flour improvers, might be worth while. The effect upon bread quality and its keeping characteristics of adding certain enzyme-containing substances to various grades and classes of flour may be of interest.

There are many potential food products that deserve attention and that may have a place in food: among these are wheat germ, bran, gluten, rice polish, corn germ, soy flour, and flour from legumes in general. And finally we know very little about the use of the rarer minerals present normally in wheat and flour and the role they play.

At the Parisian hotels for their famed continental breakfast they serve you black coffee, hot milk, and a small roll of bread which has many of the characteristics of a special kind of cement coated with a light brown lacquer. Some people claim to like it but I judge it must be rather destructive to bridge work. The French housewives carry home from the bakers a long thin hard loaf that is apparently sold by the yard. If it is dropped in the street it bothers them not a bit—they still eat it and apparently like it. The South still dotes on beaten biscuits. I'd rather have hot bread. The miner of the West swears by his sour dough. It was also quite popular among the loggers of the northern woods and many a tale has been woven about the wonderful things it can do to and for a man. In almost any difficult situation, from a broken leg to moving a boulder, some one was sure to recommend the use of sour dough, but anyway, from experience I can testify to the fact that it is filling, and any man in the mining camps who can't make a batch of sour dough slapjack is considered a tenderfoot and an outcast. During the Civil War the Confederate troops were given rations of hardtack and I've heard many an old soldier state he learned to like them, but one in particular I recall added that he liked them better after dark as then he couldn't see what was in them. All this goes to show that tastes differ, food habits differ, all of which complicate your problems.

After the lapse of a good many years I still have memories of the bread my mother used to make—warm bread from the oven with its delightful aroma. When this bread was covered with a generous quantity of butter, it was perfect in my estimation because it satisfied my youthful appetite as nothing else could do. I would be inclined to feel that this is a memory of a childish taste and perhaps it is. It merely confirms my belief that research is the answer to this and many

other problems in the cereal industry. In my opinion, bread consumption would increase by leaps and bounds if the commercial bakers pushed their research until they could turn out bread as good as we like to believe our mothers made.

And now in conclusion, I'd like to say that while I cherish the happy memory of the days when I covered my mother's bread with butter and ate it while it was hot, yet I am convinced that the bread that is produced in the good baking establishments in this country today is the best, most nutritious, and most palatable commercial bread that is produced in the world. Research made it so. And research will improve our bread still further if we give it a chance and follow the right sort of research.

ACIDITY IN CEREALS AND CEREAL PRODUCTS, ITS DETERMINATION AND SIGNIFICANCE

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(Read at the Annual Meeting, May 1938)

The Bureau of Agricultural Economics, in an effort to improve existing methods for evaluating quality of the cereal grains, has been making a study of acidity as a possible practical indication of deteriorative changes occurring in the grain.

A great many methods have been proposed for the determination of titratable acidity in cereals and cereal products. Historical reviews of this subject, particularly as it applies to flour, have been made by Brooke (1929) and by Collatz (1929).

The proposed methods may be divided into three general classes: (1) water extraction methods, (2) alcohol extraction methods, and (3) fat-solvent extraction methods. The methods most extensively employed in this country may be summarized as follows:

(1) *The A. O. A. C. tentative method (for flour).*—This method, as described in the A. O. A. C. Official and Tentative Methods of Analysis (1935), consists in digesting the flour for one hour at 40° C. and titrating the filtered extract with standard alkali using phenolphthalein as an indicator. Acidity is reported as percent lactic acid.

(2) *The Greek or Balland method.*—The Greek government has adopted as its official method for determining the acidity of flour a method by which the flour is extracted with 85% alcohol, filtered, and

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the filtrate titrated with alcoholic potash using curcuma as an indicator. Results are expressed as percent sulfuric acid.

(3) *Schulerud's (1932) method.*—The flour is digested with 67% alcohol, and the filtrate is titrated with standard alkali, using phenolphthalein. Results are expressed as milliliters of normal alkali required to neutralize the acid from 100 g. of flour.

(4) *Coleman's method.*—Coleman (1929, 1930) has used the acid value of the petroleum ether extract of wheat as a measure of the degree of soundness of the wheat and as a method for predetermining flour soundness. Results are expressed in terms of the acid number, *i.e.*, the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in one gram of fat.

(5) *The Besley and Baston (1914, 1916) method.*—This method for determining the acidity of corn is essentially an adaptation of the method of Black and Alsberg (1910), and consists in digesting the meal with 80% alcohol, filtering, diluting an aliquot of the filtrate with water, and titrating the acid with standard alkali using phenolphthalein. Results are expressed as the number of milliliters of normal potassium hydroxide required to neutralize the acid extracted from 1,000 grams of corn.

Acidities as determined by the above methods are all expressed in different terms, but even though the values are all converted to the same terms, wide differences are shown in values obtained by the various methods. Fifield and Bailey (1929) and Markley and Bailey (1931) have compared extensively the Greek method with the A. O. A. C. method. Whereas a high acidity by one method was generally associated with a high acidity by the other method, no mathematical relationship between the values obtained by the two methods was found. The authors in applying the above methods to corn of different degrees of soundness were also unable to establish any very definite relationships among the various methods.

That the failure of the various methods to give concordant or even comparable results was due to the fact that different types of acid-reacting substances were extracted by the various solvents has been suspected by several investigators. Johnson and Green (1931) have shown that the organic acids formed in flour during storage consist chiefly of long carbon chain acids soluble in ether or strong alcohol but insoluble in water. Schulerud (1932), assuming that the acids present in flour consist primarily of fatty acids and acid phosphates, has shown that strong alcohol such as is used in the Greek method fails to extract the acid phosphates, whereas water extraction methods will not account for the fatty acids. He recommends 67% alcohol

as the concentration which will extract the maximum amount of both types of acid, higher concentrations failing to extract all the acid phosphate, and lower concentrations failing to extract the fatty acids quantitatively. Schulerud also points out that water extracts contain not only the acid phosphates originally present in the flour, but acid phosphates which are formed by enzymatic action during the extraction process. The titration value of the water extract depends on the temperature and time of digestion, and at room temperature does not reach a maximum value for nearly two days. In a later paper Schulerud (1933) presents evidence to indicate that the increase in flour acidity during storage is due principally to an increase in free fatty acids regardless of the method used in determining the acidity, thus confirming conclusions previously reached by Johnson and Green (1931). It also has been shown by Kozmin (1935) and Kozmin and Alakrinskaya (1935) that this increase in the free fatty acid content of flour during storage is largely responsible for the physical changes occurring in the gluten.

Separation of Acids into Classes

In the present investigation the authors have made a further study of the nature of the acid components in various types of cereal extracts in an attempt to explain better the observed differences in the titratable acidity of these extracts.

In the case of the A. O. A. C. water extraction method, of the petroleum ether extraction method, and of the alcohol extraction methods as a group, the differences in results obtained may conceivably be due to differences in the solubilities of the various acidic substances in water, petroleum ether, and alcohol. Among the various alcoholic extraction methods, however, the differences in acidity values appear to be much greater than could be explained by the relatively small differences in the concentrations of alcohol used for extraction. For example, with both the Greek method and the Black and Alsberg method 85% alcohol is used for extraction, yet the Greek method indicates acidities often nearly double those of the Black and Alsberg method. Obviously these differences must be due to some factor other than solubility.

In the case of the Greek method, the acids are titrated directly in the 85% alcoholic solution used for extraction, whereas with the Black and Alsberg method the alcoholic solution is diluted with water to a concentration of about 14% alcohol before titrating.

In order to determine the effect on the titration value of concentration of alcohol in the solution being titrated, aliquots of a 50% alcoholic extract of a sample of damaged corn were diluted with varying quanti-

ties of alcohol and water and the resulting solutions titrated with standard alkali. The results are shown in Table I and graphically in Figure 1.

TABLE I

SHOWING THE EFFECT OF THE CONCENTRATION OF ALCOHOL IN THE SOLUTION BEING TITRATED ON THE TITRATION VALUE OF A 50% ALCOHOLIC EXTRACT OF A SAMPLE OF DAMAGED CORN

Concentration of alcohol at end-point of titration	Acidity [1]
% by volume	
86	39.3
79	39.1
75	37.6
65	34.0
56	29.8
47	27.9
33	23.3
19	18.7
10	15.9
5	14.6
2.5	14.3

[1] Acidity is expressed in terms of milligrams of KOH required to neutralize the acids from 10 grams of corn (dry-matter basis).

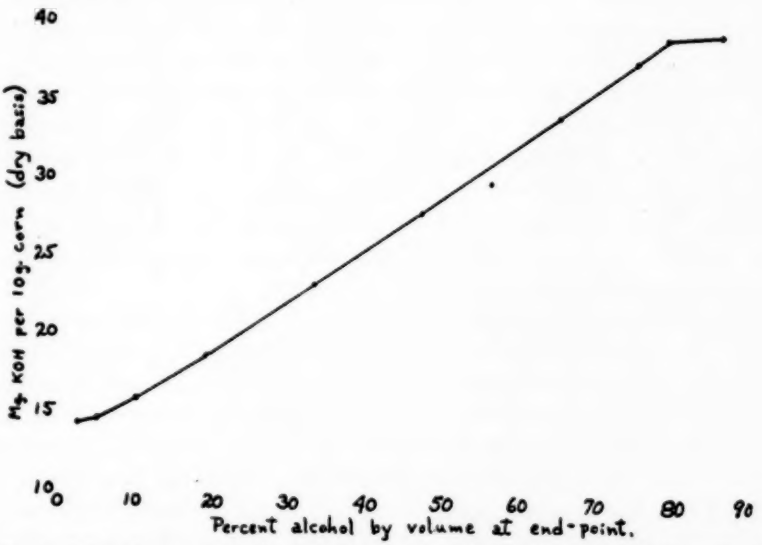

Fig. 1. Effect of concentration of alcohol in solution being titrated on the titration value of a 50% alcoholic extract of a sample of damaged corn.

Thus the apparent acidity of this particular extract when titrated in 86% alcohol is nearly three times as great as when titrated in 2.5% alcohol. Foreman (1920) has shown that in 85% alcohol the amino

groups of amino acids do not have a basic reaction and that the carboxyl groups may therefore be titrated quantitatively with standard alkali. In water solution the monocarboxylic monoamino acids react neutral. This principle has been used extensively as a convenient method for estimating amino acids and *explains the differences in apparent acidity of either alcoholic or water extracts of cereals when titrated in different concentrations of alcohol.* This fact appears to have been entirely overlooked in comparative studies which have been made on various methods for determining acidity in cereals and cereal products. In general it may be stated that the concentration of alcohol in the solution titrated is of greater significance than the concentration of alcohol in the solution used for extraction.

In order to determine the relative amounts of the various types of acidic substances extracted by different concentrations of alcohol, the following experiment was performed:

Eleven 10-gram portions of a commercial hard red winter wheat flour were shaken for 30 minutes with 100-ml. portions of neutral alcohol-water mixtures of different concentrations. The suspensions were then centrifuged and two 25-ml. aliquots of each centrifugate taken. To one of these aliquots was added sufficient neutral 95% alcohol to bring the concentration of alcohol in the mixture to 85%. To the other aliquot was added sufficient CO_2-free water to reduce the concentration of alcohol to 5%. The titration value of each solution was determined with phenolphthalein as an indicator.

A second series of eleven 10-gram portions of the same flour were extracted for 16 hours with petroleum ether in Soxhlet extractors. The free fatty-acid content of the extracts was determined, the residues were extracted with various concentrations of alcohol, and the acidities of the extracts determined in the same manner as in the case of the original flour samples.

From the data obtained it is possible to divide the acidity extracted from the original flour by each of the 11 concentrations of alcohol into three distinct fractions, the amino acid acidity, the acid phosphate acidity, and the fat acidity (Table II). This may be understood more clearly by referring to the curves in Figure 2. Curve I represents the total titratable acidity of each extract as determined by titrating the extracts of the original flour in 85% alcohol. Curve II represents similar values for the alcoholic extracts of the fat-free flour, and therefore represents total acidity minus fat acidity. Obviously the differences between the values in curve I and curve II represent the fat acidity, which is plotted as curve VI. Curve V represents the acidity of the alcoholic extracts of the fat-free flour as determined by

titration in 5% alcohol and may be considered to be essentially the curve for the acid phosphates. The difference between curve II and curve V represents the amino acid acidity, which is plotted separately as curve IV.[3]

The high values for amino-acid acidity, phosphate acidity, and total acidity in the water (0% alcohol) and 10% alcohol extracts are probably due to the presence of acids formed by enzymatic action during the extraction process, in addition to the acids already present in the flour. That this is the case is evidenced by the fact that in

TABLE II

DETERMINATION OF THE DIFFERENT ACID FRACTIONS EXTRACTED FROM A COMMERCIAL HARD RED WINTER WHEAT FLOUR BY DIFFERENT CONCENTRATIONS OF ALCOHOL

All acidity values are in terms of milligrams of KOH required to neutralize the acid extracted from 100 grams of flour (dry-matter basis).

Concentration of alcohol %	Original flour		Petroleum ether extracted flour		Amino-acid acidity C-D	Fat acidity [1] A-C
	A Acidity in 85% alcohol. Total acidity	B Acidity in 5% alcohol	C Acidity in 85% alcohol	D Acidity in 5% alcohol—"phosphate" acidity		
95	47.6	29.6	28.2	12.6	15.6	19.4
90	57.0	34.1	39.1	18.9	20.2	17.9
80	109.5	57.5	91.6	41.8	49.8	17.9
70	135.6	65.5	117.6	54.3	63.3	18.0
65	144.1	66.9	126.6	60.6	66.0	17.5
60	143.7	68.7	129.7	60.2	69.5	13.5
50	140.1	66.4	131.5	60.6	70.9	8.6
40	134.7	67.3	131.1	61.5	69.6	3.6
30	132.4	58.8	129.7	55.7	74.0	2.7
20	127.0	56.6	126.2	55.2	71.0	0.8
10	141.0	65.1	140.5	65.1	75.4	0.5
0	153.1	73.2	154.4	70.9	83.5	−1.3

[1] Fat acidity as determined by petroleum ether extraction: 19.0.

water solution the quantity of acid extracted is a function of the time and temperature of extraction, whereas in alcoholic solutions stronger than 20% no appreciable increase in acidity occurs after the first 15 minutes of digestion.

[3] It should be pointed out that the acid fraction which we are designating as "phosphate" acidity and which is represented by curve V in Figure 2 consists of certain other acid-reacting substances in addition to the acid phosphates. These additional acidic factors consist of one of the carboxyl groups of any dicarboxylic amino acids present, the acid-binding power of any peptized protein present, and possible traces of organic acids not soluble in petroleum ether. Acid phosphates, however, undoubtedly account for the major part of this fraction.

It should further be pointed out that the amino-acid fraction as represented by curve V, since it is based on the Foreman titration, does not account quantitatively for any proline or dibasic amino acids which may be present.

Considering the extracts made with alcohol concentrations of 20% or over, in which enzymatic activity appears to be inhibited, it will be observed that a maximum total acidity is obtained at an alcohol concentration of about 65%, thus essentially confirming Schulerud's observation that 67% alcohol extracts the maximum quantity of acid from flour. It should be noted, however, that whereas Schulerud's

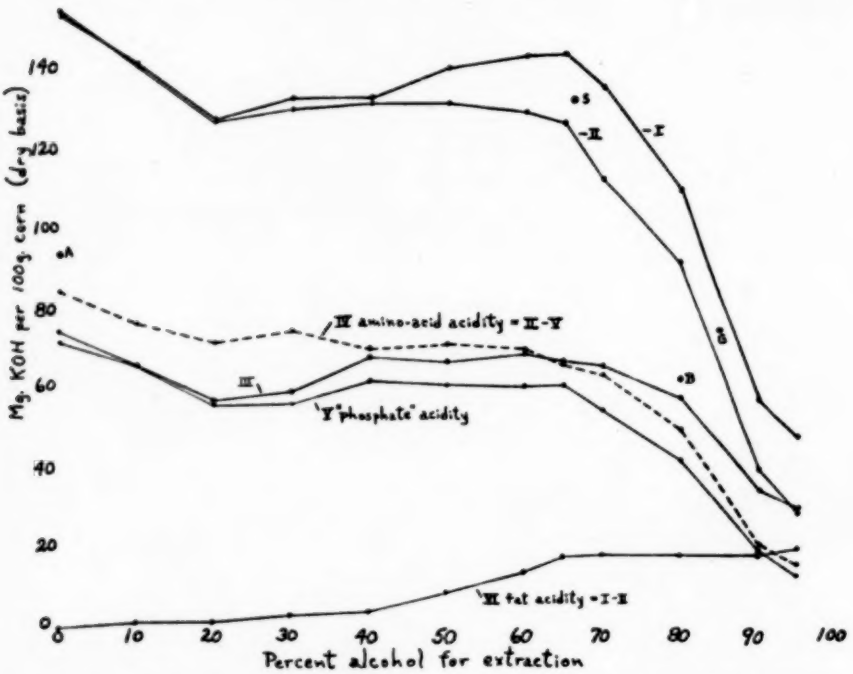

Fig. 2. Acid fractions extracted from a commercial hard red winter wheat flour by different concentrations of ethyl alcohol.

Curve I. Total acidity. Titration in 85% alcoholic solution.
Curve II. Total acidity of fat-free sample. Titration in 85% alcoholic solution.
Curve III. Acidity by titration in 5% alcoholic solution.
Curve IV. Amino acidity. II minus V.
Curve V. "Phosphate" acidity. Titration of extract of fat free sample in 5% alcoholic solution.
Curve VI. Fat acidity. I minus II.
Point A. Acidity by the A. O. A. C. tentative method.
Point B. Acidity by the Besley and Baston method as applied to flour.
Point G. Acidity by the Greek or Balland method.
Point S. Acidity by Schulerud's method.

67% alcohol extract may *contain* the maximum total acidity, this concentration of alcohol is too low to *determine* the maximum acidity. The strength of alcohol in such an extract should be increased to 85% before titration in order to account for the maximum possible amino acid content.

It will also be observed that the amino-acid content is essentially constant at a maximum value in extracts containing between 20%

and 60% alcohol, and "phosphate" acidity between 20% and 65% alcohol. Fat acidity values are nearly constant at concentrations of alcohol greater than 65% and agree very well with the value determined by petroleum ether extraction. Thus no single concentration of alcohol is capable of extracting quantitatively all three types of acidic products, when the ratio of solvent to flour commonly employed in acidity determinations is used. Alcohol concentrations between 60% and 70% will in general extract most of the acids of all three types from flours containing less than 1% of fat.

Curves similar to those shown in Figure 2 are obtained for both wheat and corn, maximum total acidity values in all samples tested being extracted with alcohol of between 60% and 70%.

The following procedure, based on the foregoing experiments, has been adopted for determining the three classes of acid-reacting compounds in corn, wheat, and flour:

Ten-gram portions of the flour or finely ground grain are extracted for 16 hours with petroleum ether in Soxhlet extractors. After evaporation of the solvent, the extracts are dissolved in 50 ml. of 1 : 1 benzene-ethyl alcohol mixture containing 0.02% phenolphthalein, and fat acidity determined by titration with standard alkali. A blank titration on the benzene-alcohol mixture is subtracted from the titration values of the extracts.

The extracted meals or flours are suspended in 100 ml. of 60% ethyl alcohol neutral to phenolphthalein in stoppered flasks. The suspensions are shaken at frequent intervals for 30 minutes and then filtered. One 25-ml. aliquot of the filtrate is diluted with neutral 95% alcohol to a concentration of 85% alcohol and titrated. A second 25-ml. aliquot is diluted with CO_2-free water to a concentration of 5% alcohol and titrated. The titration in 5% alcohol corresponds to the "phosphate" acidity and the difference between the two titrations corresponds to the amino-acid acidity.[4] Results are calculated as the number of milligrams of KOH required to neutralize the acids extracted from 10 g. of the dry grain or flour.

Significance of the Different Types of Acidity

Numerous investigators have presented evidence that deterioration in cereals and cereal products is accompanied by an increase in the titratable acidity of various types of extracts. Since most of the methods previously employed to measure acidity determine only combinations of fractions of the three types of acidities herein discussed, the present study is concerned with the relative significance of these three types of acidity as measures of soundness or deterioration.

[4] See footnote on page 585.

Fat acidity, "phosphate" acidity, and amino-acid acidity were determined on a series of 252 samples of corn. These samples were classified according to commercial grade as specified in the official grain standards of the United States, "damaged kernels" being the determining grading factor. The average values for the three classes of acidity for each commercial grade are shown in Table III.

TABLE III

AVERAGE VALUES FOR FAT ACIDITY, "PHOSPHATE" ACIDITY, AMINO-ACID ACIDITY, AND TOTAL ACIDITY OF 252 SAMPLES OF CORN CLASSIFIED BY GRADE ACCORDING TO THE FACTOR, "DAMAGED KERNELS"

Grade	Damaged kernels %	Number of samples	Fat acidity [1]	"Phosphate" acidity [1]	Amino-acid acidity [1]	Total acidity [1]
1	0 – 3.0	88	17	73	117	207
2	3.1– 5.0	46	26	75	118	219
3	5.1– 7.0	28	36	77	114	227
4	7.1–10.0	31	46	92	120	258
5	10.1–15.0	28	53	99	127	279
Sample grade	>15.0	31	125	158	170	453

[1] Acidity is expressed as the number of milligrams of KOH required to neutralize the acids extracted from 100 grams of corn (dry-matter basis)

Germination tests [5] also were made on 209 samples of corn from the same series. Average acidity values for different ranges in germinability are shown in Table IV.

TABLE IV

AVERAGE ACIDITY VALUES OF 209 SAMPLES OF CORN CLASSIFIED ACCORDING TO GERMINABILITY

Germination %	Number samples	Fat acidity [1]	Phosphate acidity [1]	Amino-acid acidity [1]	Total acidity [1]
90–100	31	15	73	116	204
80–89	54	20	69	114	203
70–79	21	26	79	121	226
50–69	29	36	80	115	231
30–49	26	42	87	120	249
10–29	21	51	104	128	283
0–9	27	117	130	158	405

[1] Acidity is expressed as the number of milligrams of KOH required to neuralize the acids extracted from 100 grams of corn (dry-matter basis).

The percentage increase in the different acid fractions corresponding to lowering commercial grade and decreasing germination is shown graphically in Figures 3 and 4, respectively.

[5] Germination determinations were made under the supervision of E. H. Toole, of the Division of Seed Investigations, Bureau of Plant Industry.

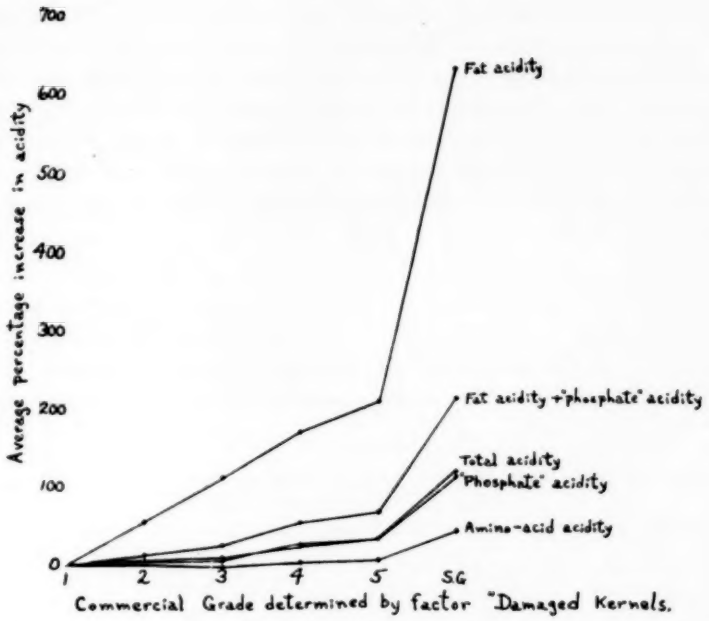

Fig. 3. Average percentage increase in acidity values of corn corresponding to lowering commercial grade.

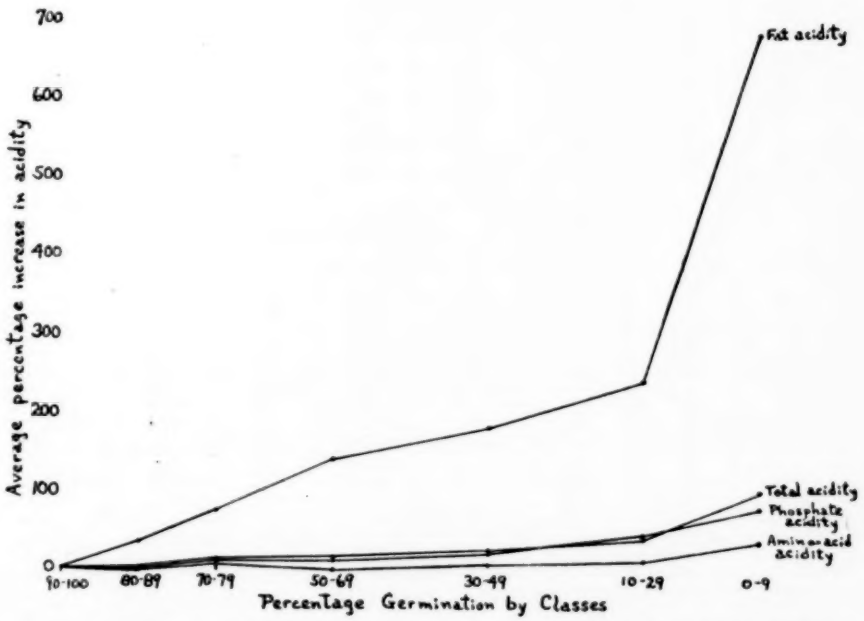

Fig. 4. Average percentage increase in acidity values of corn corresponding to decreasing germinability.

Thus it may be seen that in the case of corn, fat acidity increases at a much greater relative rate with increasing deterioration as measured both by commercial grade and by germination, than does either of the other types of acidity. It also should be noted that a significant increase in "phosphate" acidity occurs only in samples which have undergone a considerable degree of deterioration, and amino acids increase only when the deterioration is well advanced, whereas highly significant increases in fat acidity appear at very early stages of deterioration. It appears, therefore, that of the three types of acidity under consideration, fat acidity alone may be used as an index of incipient deterioration of corn. The correlation coefficient between fat acidity and the percentage of damaged kernels (as determined for grading purposes) for 245 samples was found to be + 0.90, and be-

TABLE V

DATA ON HARD RED WINTER WHEAT SAMPLES FROM BINS H-2-W AND H-1-E AT HAYS, KANSAS, 1937

Days in storage	Odor	Damaged kernels %	Germination [1] %	Fat acidity [2]	"Phosphate" acidity [2]	Amino-acid acidity [2]	Total acidity [2]
			Bin H-2-W. Moisture: 14.45%				
1	N [3]	0.0	82	21.2	57	106	184
7	N	0.2	79	20.8	60	107	188
13	N	0.1	80	22.3	60	105	187
20	N	0.2	60	23.3	59	108	190
27	N	0.2	66	24.6	62	104	191
34	N	1.0	53	26.4	63	105	194
48	N	5.0	17	33.0	69	103	205
55	M [4]	5.7	18	40.0	75	103	218
			Bin H-1-E. Moisture: 15.35%				
1	N	0.0	73	24.6	57	118	200
7	N	0.2	60	27.6	58	116	202
13	V.S.M. [5]	4.0	47	33.1	63	115	211
16	M	3.8	46	33.9	63	115	212
20	M	4.3	24	44.9	66	116	227
27	N	4.5	16	49.9	72	114	236
34	M	5.4	11	52.0	74	114	240
41	M	7.0	7	55.6	78	112	246
48	M	4.7	8	53.9	82	111	247
55	M	5.0	9	54.6	83	112	250

[1] Normal plus abnormal germination.
[2] In terms of milligrams of KOH required to neutralize the acids from 100 grams of wheat (dry matter basis). Samples were analyzed from 3 to 5 months after removal from bin, during which time acidity values had probably increased.
[3] Normal.
[4] Musty.
[5] Very slightly musty.

tween fat acidity and the logarithm of the percentage germination the coefficient was − 0.85 for 213 samples.

The Bureau of Agricultural Engineering is conducting an extensive wheat-storage project, the purpose of which is to determine the most suitable types of farm bins for the storage of wheat and the maximum moisture limits at which wheat may be stored safely in such bins. The Bureau of Agricultural Economics is cooperating in this project by

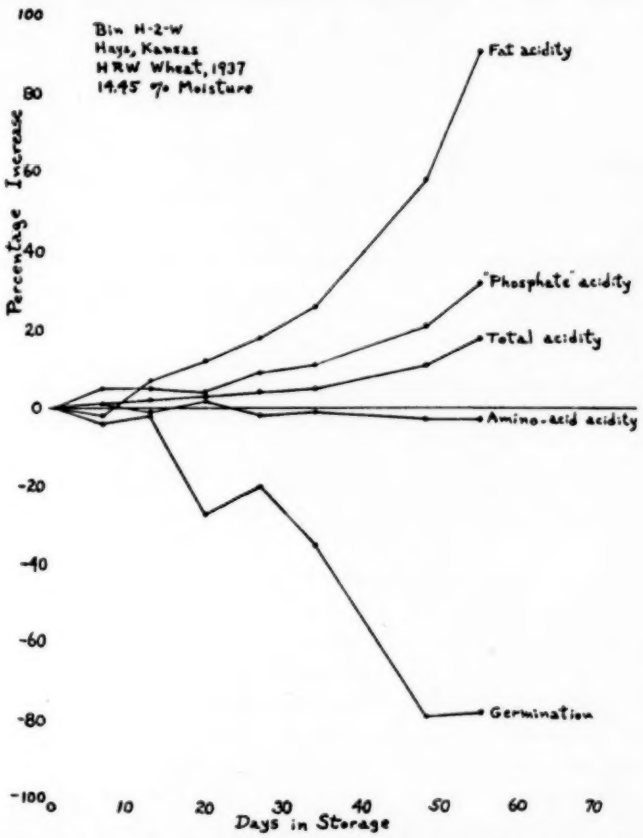

Fig. 5. Percentage change in acidity values and germination of hard red winter wheat stored at 14.45% moisture. Bin H–2–W; Hays, Kansas; 1937.

grading and determining fat acidity values on samples drawn from the experimental bins.

The data on samples of hard red winter wheat taken at intervals from two different 250-bushel plain metal storage bins located at Hays, Kansas, are given in Table V. The increase in the various acid fractions and the decrease in germination on a percentage basis are shown graphically in Figures 5 and 6. As in the case of corn, the percentage

increase in fat acidity during deterioration is much greater than the percentage increase in either of the other types of acidity. Also, significant increases in fat acidity occur at a much earlier stage of deterioration than do increases in either "phosphate" or amino-acid acidity. No increase whatever in amino acids was apparent during the storage period covered by these experiments.

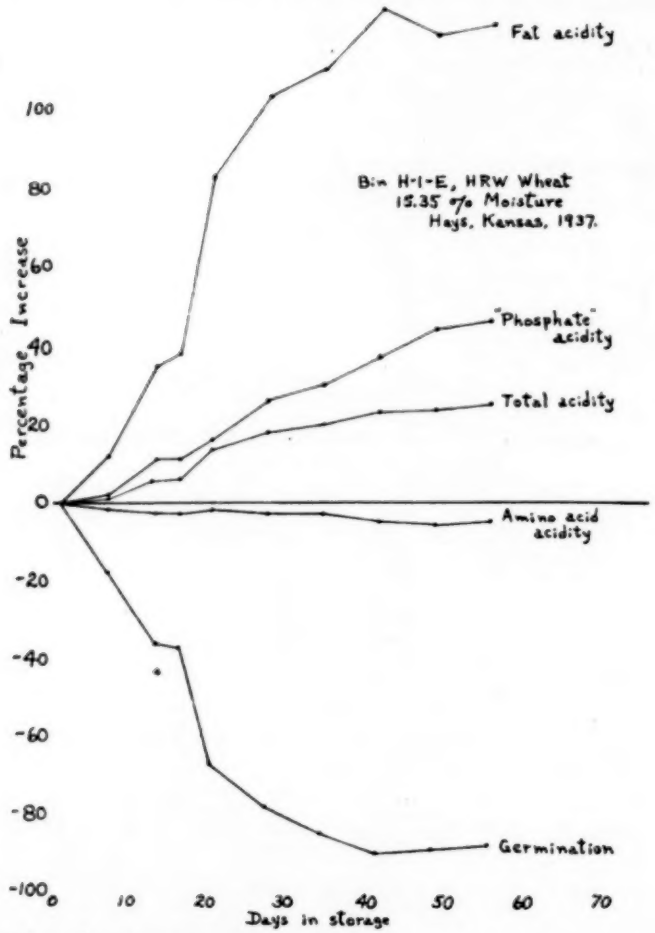

Fig. 6. Percentage change in acidity values and germination of hard red winter wheat stored at 15.35% moisture. Bin H–1–E; Hays, Kansas; 1937.

In the case of both wheat and corn the fat acidity value based on a definite weight of grain appears to be a more reliable index of soundness than does the acid value of the fat, since the fat from unquestionably sound grain of high fat content nearly always has a lower acid value than does the fat from grain of low fat content.

It should be noted that whereas acidity as determined by the Greek method or by Schulerud's method is due largely to the presence of amino acids, this amino acid acidity has little if any significance as a measure of deteriorative changes in the cereal.

It should be pointed out further that since the amino acids (other than the dicarboxylic amino acids) present in a cereal do not show an acid reaction except in alcoholic solutions, they have no appreciable effect either on the hydrogen-ion concentration or the titratable acidity of aqueous extracts, suspensions, or doughs. It seems doubtful, therefore, whether such amino acids should be included in any determination designed to measure true titratable acidity. It also has been observed, in the case of wheat, that the amino acid content is closely related to the protein content of the grain. Thus if titratable acidity is to be used as a measure of relative soundness among wheats of different protein content, the inclusion of amino acids in the acidity determined will lead to confusion, and will decrease the reliability of the method as an index of soundness.

Summary

The acidic substances present in cereals and cereal products may be divided into three principal classes: (1) free fatty acids, (2) acid phosphates, and (3) amino acids. These acids are present in small quantities in all normal grain and under certain conditions increase in amount chiefly by virtue of the enzymatic hydrolysis of fats, phytin, and proteins, respectively.

The determination of titratable acidity has frequently been proposed as an indication of deteriorative changes occurring during storage. Many methods have been proposed for determining acidity, but generally speaking the various methods not only fail to yield concordant results but fail to yield results which are even approximately proportional. This lack of agreement between methods appears to be due to the fact that the various methods determine varying fractions of the three classes of acidic substances present. Since the ratios among the different types of acid are not constant, attempts to show mathematical relationships between acidity values determined by different methods are largely futile.

None of the four methods for determining titratable acidity in cereals and cereal products included in the A. A. C. C. Cereal Laboratory Methods appears to be based on a fundamental concept of the nature of the acids being determined.

The A. O. A. C. water extract method (for flour) determines acidity due primarily to acid phosphates. The amino acids are present in the extract but are not determined by the titration procedure used. Fatty

acids are neither extracted nor determined. The principal weakness of the method appears to be that not only the acid phosphates originally present in the flour but also acid phosphates formed by enzymatic action during the extraction process are determined.

The Greek or Balland method (for flour) extracts and determines essentially all of the free fatty acids plus varying fractions (generally less than half) of the acid phosphates and amino acids.

Schulerud's 67% alcohol method (for flour) extracts the major part of all three types of acids but fails to determine quantitatively the amino acids extracted.

The Besley and Baston method (for corn) extracts essentially the same acids as the Greek method, but determines only a small and variable fraction of the amino acids extracted.

A study of the individual acidity fractions in 246 samples of corn and of the same fractions in wheat undergoing deterioration in storage reveals the fact that only the fat acidity increases significantly during the early stages of spoilage. Acid phosphates increase only after the deterioration is moderately advanced, and amino acids show an increase only in badly damaged grain.

Since the amino-acid content is a function of the protein content of the grain or flour, its inclusion as a part of the acidity determined by any method designed to be an index of soundness or deterioration is undesirable.

Fat acidity alone appears to be a more reliable index of soundness in grain than either of the other types of acid present, or than any combination of these acid fractions as determined by any of the commonly used methods.

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SOME OBSERVATIONS ON THE FRACTIONATION OF WHEAT FLOUR PROTEIN IN THE LIGHT OF COLLOID CHEMISTRY

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(Read at the Annual Meeting, May 1937)

Recent work on the protein material of wheat flour has indicated that the classification of the proteins as laid down by the Physiological and Biochemical Committees on Protein Nomenclature (1908) is untenable, at least in so far as the protein of wheat flour is concerned.

The classification is based chiefly on differences in the "solubility" of the so-called protein "individuals" in various "solvents." Since proteins are colloidal in nature, it does not necessarily follow that dispersion of a protein in a solvent is solubility at all, in the sense that the term is used in physical chemistry for crystalline systems. Protein, as it exists in the ripe wheat kernel, or in wheat flour, is probably a dried-up hydrogel. When water is added, the protein becomes hydrated and the system becomes either a sol or a gel, depending on the amount of water adsorbed. The molecule or particle then shows the properties of a typical emulsoid. Colloidal systems may be dispersed in a number of ways, among which peptization is probably the most important. In the case of the proteins, recent work reported in the literature has shown that it is very probable that *solubility is synonymous with peptization*, and that the action of the so-called "protein solvents" is merely the breaking down of large complexes into smaller fractions, which then remain in suspension as colloidal sols. Certainly the pioneer work of Gortner, Hoffman, and Sinclair (1928, 1929) and more recent studies by various investigators have shown that the term "solubility" has a wider meaning when applied to the proteins, than is usually applied to it.

Colloidal systems fall into two general classes. These classes have been designated suspensoids or lyophobic systems, and emulsoids or lyophilic systems. Many of the properties of the protein of wheat flour are those of typical lyophilic systems, and many of the reactions of the proteins which are difficult to explain on the basis of the classical chemical reactions are readily interpreted in the light of the characteristics of colloidal systems.

It is a well known fact that the H^+ or the OH^- ions cause wheat protein to become highly hydrated, and at the same time to pass into the sol state, in which state the system *has the appearance* of being in true solution. Dispersion of flour proteins in dilute acid or alkali solutions is a process of solvation, and thus is accompanied by hydration of the dispersed protein. Any condition which will cause the dehydration of such highly hydrated particles will cause flocculation and "precipitation" of the protein.

The state of hydration of protein systems may be measured in various ways. Fischer (1921) measured the amount of swelling of the substance during hydration. This method has been applied to wheat flour protein by Berliner and Koopman (1929). Hofmeister (1890) measured the increase in weight of the substance, which method was also used by Upson and Calvin (1916) with gluten. Newton and Cook (1930) applied cyroscopic methods. These three methods are capable of giving only very rough measurements, and whenever possible a fourth method is used—that of measuring the viscosity of the dispersed sol. Changes in the degree of hydration may be most easily and accurately followed by changes in the viscosity of the system.

Gortner (1929, page 376) stated that if solubility of the proteins is a peptization process, then the phenomenon should take place according to the diagram shown in Figure 13. At 'B' that portion of the total protein dispersed by the ions in question is at its maximum state of hydration. On the addition of further amounts of the dispersing agent, the protein particles become dehydrated, until at 'C' they have so far lost their water of hydration that they approach the suspensoid state, and therefore precipitate.

Rich (1936) showed that the "solubility" of wheat flour proteins in salt solutions produces curves of essentially the same form. It was shown, also, in this paper that the proportion of the total protein dispersed and hydrated to a sufficient state that it could be decanted from the remainder (this was represented by the height of the curves) depends on the nature of the salt (*i.e.*, on the ions of which the salt was composed). The range in the dispersing effect of the ions on the wheat-flour protein was more forcibly shown by Gortner, Hoffman, and Sinclair (1929). They showed that a molar solution of KF dispersed only about 19% of the total protein, whereas a molar solution of KI dispersed as much as 64% of the total. That this effect is not specific for wheat proteins only was shown by Staker and Gortner (1931), who demonstrated that many other seed proteins behave in a similar manner to different salt solutions.

Until recent years complete dispersion of wheat-flour protein has been accomplished only by dilute solutions of acids or bases—or speaking accurately, by the H^+ and the OH^- ions. In the presence of these ions, the protein quickly hydrates—becomes a swollen mass and finally becomes a more or less turbid sol. Several investigators have shown that the dispersed and hydrated particles are not all of the same dimensions. Blish and Sandstedt (1929) point out that even with dilute acetic acid solution part of the protein is dispersed into such large particles that they cannot be decanted or centrifuged free from the starch of the flour, so that dispersions free from starch can only be obtained by using crude gluten as the starting point. Blish (1936) also points out that from 10 to 12% of the protein dispersed in dilute acetic acid or in sodium salicylate solution is in large enough particles that it may be thrown out by the supercentrifuge running at 30,000 to 40,000 r.p.m.

Several investigators have stressed the fact that very weak solutions of acids must be used to effect complete dispersion. In actual practice, acetic acid is usually chosen, and experiment has shown that solutions of about 0.05 normal are the proper concentration. The correct concentration is important because, as Upson and Calvin (1916) have shown, after a certain concentration of acid is reached, gluten will no longer go into "solution," because dehydration begins, and crude gluten placed in such solutions actually loses water and become still more "insoluble."

The proper concentration of acid or alkali for maximum dispersion can be found by reference to the work of Sharp and Gortner (1923). They showed that wheat flour protein is at its maximum state of hydration at concentrations of hydrogen-ions equal to pH = 3 and to pH = 11. A concentration of pH = 3 is reached in the case of acetic acid at a concentration of about 0.04 normal so that the concentration that has arbitrarily been chosen is approximately correct. Their curves show that after the maximum points of hydration are reached, dehydration begins, until at about pH = 1 and 13 the protein is at the same stage of hydration as it was before acid or alkali was added. At these points precipitation of the proteins is complete, resulting in a decrease in the viscosity of the suspension equal to that of the original flour in water suspension.

The protein, after having been dispersed in weak acid solution, can be re-precipitated by either (*a*) neutralization, *i.e.*, removal of the H^+ or OH^- ions, or (*b*) the so-called "salting-out" process, in which the addition of small amounts of suitable inorganic salts causes precipitation. It is believed that the salting-out process is due to a dehydration of the hydrated particles to a point which Kruyt and

van Klooster (1930) call the "critical hydration point." At this point the shell of hydrated water is small enough that the particle approaches the suspensoid state and hence is so unstable that flocculation and precipitation are the result. It is also a matter of common knowledge that neutralization need not be complete to cause precipitation. Kruyt designates the point where precipitation by removal of H^+ or OH^- ions is accomplished, the "critical potential point" indicating that the potential at the interface between the particles and the dispersing medium is lowered so far as to render the particle unstable.

Many of the methods of isolating proteins consist of "salting out" the dispersed protein. The physiologists have adopted this method of isolating the salt-soluble protein fractions of animal origin. As an example, Porges and Spiro (1902), using Na_2SO_4 as the salting-out agent, reported the existence in blood serum of the following fractions:

Fibrogenin precipitated at 30% of saturation with Na_2SO_4.
Eu-globulin precipitated at 28–36% of saturation with Na_2SO_4.
Pseudo-globulin I precipitated at 33–42% of saturation with Na_2SO_4.
Pseudo-globulin II precipitated at 40–50% of saturation with Na_2SO_4.
Albumin precipitated at 50–100% of saturation with Na_2SO_4.

Lustig (1930), by using several salts, isolated three eu-globulins, two pseudo-globulins and three albumins from blood serum, while Block (1933) reported that blood serum contains at least six globulins and three albumins. It is pertinent to note that investigators in this field are not at all agreed as to the number and identify of the protein fractions which can be isolated from any given source by fractional precipitation with salting-out procedures.

Jones and his associates, in a long series of papers published between 1916 and 1923 in the Journal of Biochemistry, adopted the method used by the physiologists, and isolated from that fraction of the protein "soluble" in dilute NaCl solutions of a number of seeds, two fractions, which were named α and β globulins. Carrying over this method to the glutelins of seeds, Csonka and Jones (1927) fractionated, by salting out with different concentrations of ammonium sulphate, two "glutelins" of wheat flour from a dispersion of this protein in 0.2% NaOH solution.

The first fraction, named α glutenin, and comprising about $\frac{7}{8}$ of the total glutenin, precipitated at about 0.02 of saturation with ammonium sulphate, while the so-called β glutenin, comprising about $\frac{1}{8}$ of the total, separated at about 0.17 of saturation. A small amount of a third fraction, which they believed to be part of the globulin which had escaped extraction during the preliminary preparation of

the glutenin, precipitated at 0.3 of saturation. Their data are shown graphically in Figure 1, and are interesting from their resemblance to the curves shown later in this paper.

Since dispersion and re-precipitation are accomplished with such small concentrations of acids and alkalis and salts, and since the rate of the reaction with increase of concentration of these reagents is so rapid, direct measurement of the reaction such as was done with salt solutions by Rich (1936) is impossible. The curve in Figure 1 is

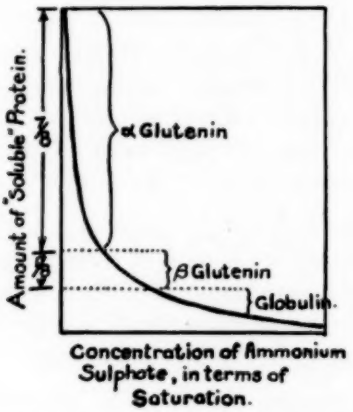

Figure 1. The amount of protein salted out of a dispersion of gluten in dilute KOH solution by ammonium sulphate. Drawn from the data of Csonka and Jones (1927).

very rough and is drawn from three points only. However, since dispersion and hydration are so closely linked, and since hydration can be followed with the viscosimeter, the reaction is easily and accurately followed by viscosimetric methods.

In the present paper some of the reactions of the proteins of wheat flour are studied by this means.

Experimental

Table I gives the ash and protein contents (calculated to a 13.5% moisture basis) of three different flours used.

TABLE I

ANALYSIS OF THE FLOURS USED IN THE EXPERIMENTS

Flour number	Ash content	Protein content
1	0.34%	13.30%
2	0.70%	18.00%
3	0.91%	18.10%

The data in Table II and Figures 2, 3 and 4 were obtained by dispersing the total protein of the three flours in dilute lactic acid and subsequently salting out the protein by the addition of different salts. Three salts were used, sodium chloride, magnesium sulphate and ammonium sulphate. The salting-out process was followed by measuring the relative viscosity of the suspensions after successive additions of the salt solutions.

TABLE II

THE EFFECT OF SALTS ON THE HYDRATION CAPACITY OF WHEAT FLOUR PROTEINS IN THE PRESENCE OF LACTIC ACID

	Effect of NaCl			Effect of $MgSO_4$			Effect of $(NH_4)SO_4$		
Flour number	1	2	3	1	2	3	1	2	3
C.c. of M/4 salt solution added	Viscosity								
0	77	38	20	75	38	21	79	39	21
0.5	—	—	—	—	—	—	48	23	13
1	59	29	20	50	24	12	25	14	8
1.5	—	—	—	—	—	—	15	10	5
2	47	23	13	33	15	9	10	5	3
3	35	18	11	23	9	7	4	2	2
4	25	15	10	15	6	6	—	—	—
5	20	12	10	11	5	4	2	2	2
6	17	10	7	7	4	2	—	—	—
7	10	6	3	6	—	—	—	—	—
8	8	—	—	—	—	—	—	—	—

A Wallace and Tiernan viscosimeter was used. The flour samples were weighed to contain 2 g. of protein so that differences in the protein content of the flours did not affect the results. By so doing, the samples from different flours contained different amounts of starch and other components. However, since starch does not affect the viscosity readings of the suspensions at the concentrations used, this method is not objectionable. The samples were allowed to stand in 100 c.c. of distilled water for 30 minutes at 22° C. At the end of this period the suspension was poured into the viscosimeter cup and an amount of lactic acid solution, which had previously been found to produce the maximum viscosity, was added. The suspension was then stirred and the viscosity immediately measured.

Although acetic acid has usually been chosen as the dispersing agent for wheat-flour proteins, lactic acid was used in this study because Sharp and Gortner (1923) showed that the pH of suspensions containing it does not go beyond the optimum pH of 3. The suspensions can, therefore, be brought to their optimum point of hydration without constant checking of the pH of the suspensions. There were

added, in increments of one cubic centimeter, 0.25 molar solutions of the salts.

The three salts differed widely in their dehydrating power on the protein of the flours. Ammonium sulphate reduced the viscosity to nearly zero at a molality of about 0.01, while the other salts would require much higher concentrations to effect the same results. This

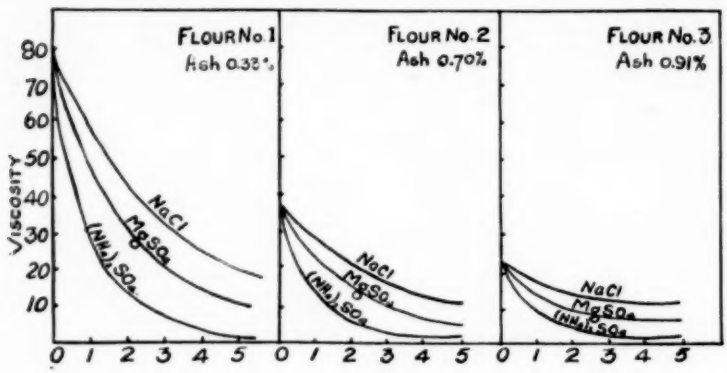

Figure 2 Figure 3 Figure 4

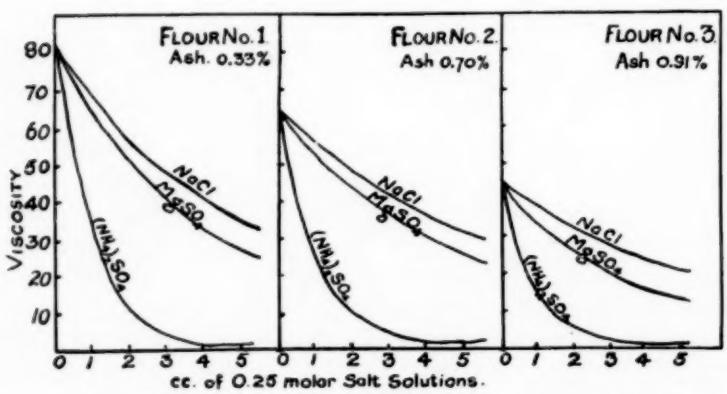

Figure 5 Figure 6 Figure 7

Figures 2 to 7. The effect of salts on the hydration of wheat flour (as measured by the viscosimeter) in the presence of lactic acid and in potassium hydroxide.

is in agreement with the fact that ammonium sulphate is the most effective salting out agent for proteins. The concentration necessary is very small and of the same order as the concentration that has been used to salt out flour protein which had previously been dispersed in dilute acid or alkali solutions.

Table II and Figures 2, 3 and 4 present the data for an experiment in which the flours were dispersed in lactic acid. Table III and

Figures 5, 6 and 7 give the data for an experiment in which the suspensions were brought to a state of maximum viscosity with KOH before the salt solutions were added. The results were similar in both experiments.

In these experiments suspensions of the whole flour were studied, so that *all* of the "fractions" contained in the flours could be studied. The data, are, however, conditioned by the naturally occurring electrolytes in the flours. In order to overcome this effect, and also in order to study the "gluten proteins" in particular, these experiments were repeated, using leached suspensions of the flours.

TABLE III

THE EFFECT OF SALTS ON THE HYDRATION CAPACITY OF WHEAT FLOUR PROTEINS IN THE PRESENCE OF POTASSIUM HYDROXIDE

	Effect of NaCl			Effect of $MgSO_4$			Effect of $(NH_4)SO_4$		
Flour number	1	2	3	1	2	3	1	2	3
C.c. of M/4 salt solution added	Viscosity								
0	85	66	44	85	65	44	85	65	45
0.5	—	—	—	—	—	—	60	46	23
1	70	58	38	68	55	34	38	26	14
1.5	—	—	—	—	—	—	10	8	5
2	58	50	35	54	46	30	4	2	2
3	48	46	28	43	38	22	—	—	—
4	40	37	24	35	32	16	2	2	2
5	35	32	20	28	26	12	—	—	—
6	30	28	18	23	22	9	—	—	—
7	28	25	15	19	18	8	—	—	—
8	23	22	12	16	15	6	2	2	2

The flours were leached according to the method outlined by Sharp and Gortner (1923). Samples of the flours, weighed to contain two grams of protein, were made into a smooth suspension with 100 c.c. of distilled water. Nine hundred additional cubic centimeters of distilled water were then added. This was allowed to stand with occasional shaking for 45 minutes, after which time it was decanted, and the liquid discarded. The residue was again extracted with 500 c.c. more of water for 15 minutes, decanted, and the residue made up to 100 c.c. The final suspension was poured into the viscosimeter and readings taken in the usual manner. By this procedure, most of the "non-gluten" fractions were removed, along with most of the soluble electrolytes. No attempt was made at this time to wash out *all* of the "non-gluten" fractions. The data obtained for suspensions dispersed in lactic acid are given in Table IV. Similar data for KOH

TABLE IV

THE EFFECT OF SALTS ON THE VISCOSITY OF LEACHED FLOUR-IN-WATER SUSPENSIONS IN THE PRESENCE OF LACTIC ACID

	Effect of NaCl			Effect of $MgSO_4$			Effect of $(NH_4)SO_4$		
Flour number	1	2	3	1	2	3	1	2	3
C.c. of M/4 salt solution added	Viscosity								
0	192	185	168	188	190	170	188	195	167
0.5	—	—	—	—	—	—	112	118	100
1	119	119	106	106	102	88	39	55	45
1.5	—	—	—	—	—	—	22	28	24
2	83	82	62	62	58	50	12	17	16
3	64	63	57	35	35	34	5	6	6
4	44	46	44	21	22	23	2	3	3
5	34	28	34	17	15	18	2	2	2
6	26	22	28	11	9	12	—	—	—
7	20	17	21	14	8	12	—	—	—
8	16	15	18	12	8	10	—	—	—
9	12	12	14	10	8	8	—	—	—
10	12	10	12	8	7	7	2	2	2

suspensions are given in Table V. The data for flour No. 1 are shown graphically in Figures 8 and 9.

It will be seen that the curves are very similar to those obtained from non-leached suspensions. They are regular, exponential curves,

TABLE V

THE EFFECT OF SALTS ON THE VISCOSITY OF LEACHED FLOUR-IN-WATER SUSPENSIONS IN THE PRESENCE OF POTASSIUM HYDROXIDE

Flour number	1	2	3	1	2	3	1	2	3
C.c. of M/4 salt solution added	Viscosity								
0	230	250	245	230	240	220	220	240	225
0.5	—	—	—	—	—	—	93	140	93
1	120	202	165	86	142	138	10	35	22
1.5	—	—	—	—	—	—	6	10	22
2	72	130	100	40	83	86	3	3	2
3	49	93	70	21	48	56	2	2	2
4	36	68	50	17	37	28	—	—	—
5	28	52	38	11	28	27	—	—	—
6	22	41	30	8	22	20	—	—	—
7	18	33	26	7	20	18	—	—	—
8	14	26	21	6	18	17	—	—	—
9	14	24	18	6	17	16	—	—	—
10	13	23	17	6	17	15	2	2	2

with no indication that two or more different fractions were present in the suspensions. With ammonium sulphate a very large proportion of the protein was precipitated from KOH dispersions by making the

system 0.005 molar with respect to the salt. These curves are very similar to those that may be drawn from the data of Csonka and Jones (1927) and which are illustrated in Figure 1.

From the curves illustrating these data it is evident that the fractionation of protein dispersions at certain salt concentrations is decidedly an arbitrary procedure. The curves are regular and smooth, and there is no indication of the point at which a fraction should be

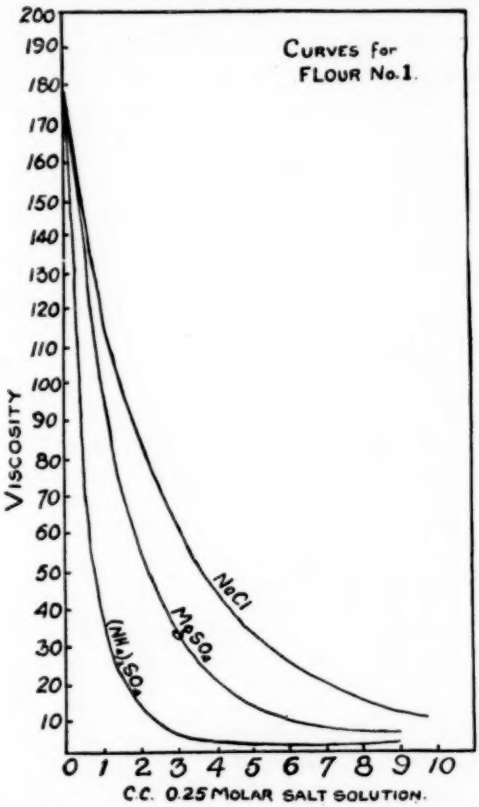

Figure 8. The influence of salts on the hydration of wheat flour protein dispersed in acid solution.

isolated. It is interesting to note that although Jones and his collaborators, in their studies of the salt-soluble proteins, filtered off the protein precipitated at certain concentrations of ammonium sulphate, they reported that the dispersions changed in the order: transparent—opalescent—turbid—distinct flocculation as the concentration of salt was increased. When, in their opinion, flocculation became distinct enough, they filtered off the precipitate, and the fractionation was continued on the filtrate by the addition of more salt. The procedure

outlined for the study of the navy bean by Waterman, Johns, and Jones (1923) is typical of their procedure. To the dispersion in 2% NaCl solution, solid ammonium sulphate was added. The precipitate obtained at 0.30 of saturation with ammonium sulphate was called an α globulin. The protein precipitated between 0.30 and 0.50 was *believed to be a mixture* of α and β globulins, and consequently *was*

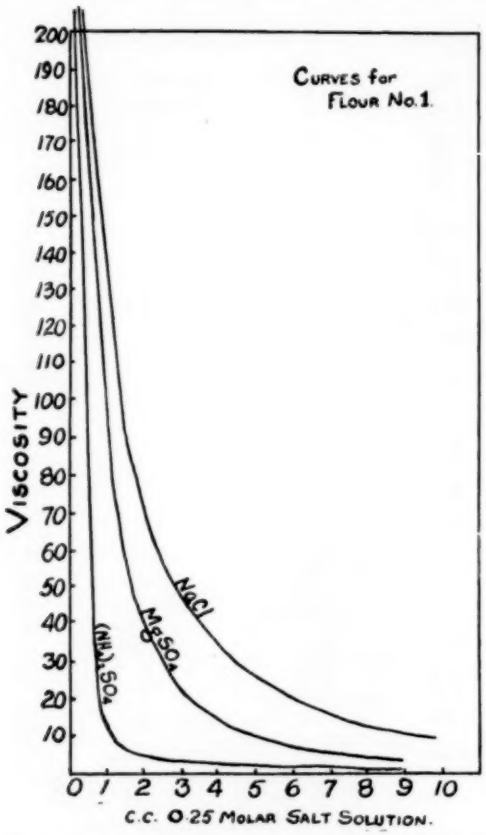

Figure 9. The influence of salts on the hydration of wheat flour protein dispersed in alkaline solution.

discarded. The protein precipitated at 0.80 of saturation was collected and called a β globulin.

Table VI and Figure 10 include data obtained by neutralizing the lactic acid in the suspension of flour with 0.25 normal KOH. Since 5.0 c.c. of lactic acid were present, 20 c.c. of the KOH solution would have been required for complete neutralization. Nevertheless, the viscosity was reduced from 195 to 8 after the addition of only 10 c.c. of KOH. The KOH curves followed the NaCl curve very closely.

Moreover, the data indicate that approximately the same curve is obtained even if the acid concentration is varied from 3 c.c. to 8 c.c. Kruyt and van Klooster (1930) state that complete removal of the charge of a suspensoid or complete removal of the hydrated water is not necessary for flocculation. The point at which the hydrated water shell or the charge is lowered enough to cause flocculation was termed the critical hydration or the critical potential, respectively.

The above data indicate no justification for discarding any part of the flocculated protein, whether it be precipitated by salting out or by neutralization procedures. These experiments do not prove the existence or the non-existence of a few protein individuals in the system. On the other hand, they do indicate that the number of

TABLE VI

EFFECT OF NEUTRALIZING THE ACID CAUSING HYDRATION OF PROTEINS

Amount of 1.0 N lactic acid used	3 C.c.	5 C.c.	6 C.c.	8 C.c.
C.c. of N/4 KOH added	Viscosity			
0	178	195	195	196
0.5	145	145	150	150
1	105	112	122	130
1.5	90	95	95	107
2	75	80	85	90
3	52	55	65	68
4	38	42	46	50
5	28	30	36	40
6	22	25	28	31
7	18	20	22	25
8	15	16	18	22
9	12	14	18	20
10	10	12	15	20

so-called "fractions" which can be isolated, either by salting out or by neutralization procedures, are limitless—the number depending only on the arbitrary adoption of salt or neutralization agent concentrations. To this may be added the nature of the dispersing agent, because Gortner, Hoffman, and Sinclair (1928, 1929), Staker and Gortner (1931), Cook and Alsberg (1931) and Rose and Cook (1934) showed that the amount of wheat flour protein dispersed by salt solutions is largely dependent on what salt is used as the dispersing agent.

Sandstedt and Blish (1933) visualized a few fractions, represented graphically as *broad plateaus* at different levels, and connected by sloping terraces, although they stated in the same paper that "there

is no established limit as to the possible minor variations in the number . . . of protein fractions by this method."

The data illustrated in Figures 2 to 7 are similar to those reported by McCalla and Rose (1935), who salted out the gluten dispersed in a 12% sodium salicylate solution by means of $MgSO_4$. Their data also produced a continuous curve with no breaks or plateaus, while

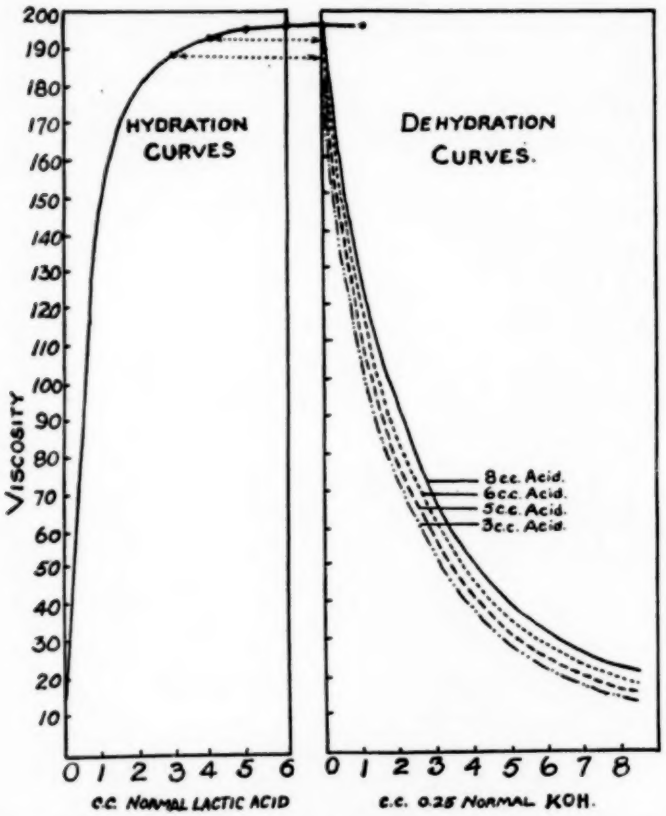

Figure 10. The dehydration of wheat flour protein (which has been dispersed and hydrated by lactic acid) by neutralization of the acid.

the "fractions" which they isolated at certain arbitrary concentrations of $MgSO_4$ varied progressively and uniformly in chemical composition as well as in physical characteristics.

The Effect of Magnesium Chloride on Protein "Solubility"

Gortner, Hoffman, and Sinclair (1928, 1929) showed that the halides of the alkaline earths, and more particularly the chlorides and bromides of Ca, Sr and Mg, apparently had different effects than the

other salts on the dispersion of wheat flour proteins. With these salts the amount of protein extracted from the suspension of flour increased with salt concentration up to 2.0 normal solution, whereas the larger concentrations of other salts extracted less than the more dilute solutions.

Henderson, Fenn, and Cohn (1919) were apparently the first to report the peculiar behavior of $MgCl_2$. They found that while Na_2SO_4, etc., lowered the "viscosity" of wheat flour doughs, $MgCl_2$ increased it, and that the viscosity became greater as the concentration of $MgCl_2$ was increased. Tague (1925) reported some evidence that $MgCl_2$ solution "dissolves" considerable quantities of "purified gliadin" and that, contrary to the effect of other salts, its "dissolving" power increased with concentration. Sharp and Herrington (1927) also showed that a 10% solution of $MgCl_2$ extracted more protein than a 5% solution. For all other salts used in their study, the reverse was true.

A study of the effects of higher concentrations of $MgCl_2$ on the amount of protein extracted from a flour was conducted by the procedure outlined by Rich (1936). It was found that a 0.25 molar solution extracted about 26% of the total protein from the flour and that 1, 2, and 3 molar solutions all extracted about 28% to 30% of the total protein, instead of decreasing amounts as would be expected. It was also noted that the "solutions" more concentrated than 1 molar were very viscous and translucent. While endeavoring to discover the effect of KI solutions it was also noted that protein "solutions" obtained with KI solutions beyond one molar were also very viscous, and that the amount of protein extracted, likewise, did not decrease with higher concentrations of KI. Its action, therefore, is apparently similar to magnesium chloride.

Reychler (1920), Ostwald and Frankel (1927) and Mangels and Bailey (1933) showed that many of the reagents which disperse wheat flour proteins also disperse starch. Just as in the case of protein peptization, these substances could be arranged in the order of the lyotropic series.

Viscosity studies show that the effect of KI, $MgCl_2$, and the other alkaline earth salts on the hydration of flour proteins is the same in acid solutions as that of all other salts studied, *i.e.* increased concentration of salt results in a decrease in hydration. It is quite probable that the effect on the dispersion of the protein in neutral solution also obeys the general rule. However because these salts are also good hydrating agents for starch, the starch of the flour, too, is affected, and the increased amount of protein extracted by decantation or centrifugation from such dispersions is *not* due to increased dispersion of the protein, but because the swollen (hydrated) starch grains hold the protein

particles from reaching the bottom of the flask. The sample taken for protein determination, therefore, contains not only the dispersed protein, but also a certain amount of hydrated starch grains together with some dehydrated protein particles which have become entangled and have not been able to settle or centrifuge out.

Since the starch-gelatinizing power of salts such as KI, $MgCl_2$, and in general salts which have a high protein-peptizing action, increases rapidly with solutions stronger than 1.0 molar, they are of little value for studying protein reactions unless washed gluten is taken for the starting point. When this is done, that fraction, of course, dispersed by the wash water, is lost before the experiment begins.

The Influence of Temperature on the Salting Out of Proteins from Dilute Acid-Alcohol Suspensions

Johnson (1927) showed that the viscosity of acidulated flour suspensions increased with temperature.

Sandstedt and Blish (1933) outlined a method by which the gluten proteins, dispersed in acetic acid—alcohol solution, could be thermally fractionated by salting-out procedures. To the dispersion a small quantity of a salt was added. As the suspension was cooled protein precipitated out. Progressive lowering of the temperature resulted in progressive precipitation of additional protein "fractions." The different "fractions" could, of course, be collected. With certain variations in procedure a large number of such fractions could be isolated. As a result of their study, Sandstedt and Blish (1933) came to the conclusion that, although there was apparently no established limit to the number of protein "fractions" obtainable by this method, for *all practical purposes* they could be grouped into three main fractions, namely, gliadin, mesonin and glutenin.

It was believed that this phenomenon could be followed with the viscosimeter. Accordingly, samples (containing 2 g. of protein) of flour No. 3 were first leached with distilled water. This removes very little of the gliadin fraction. After the final decantation the remaining suspension was made up to 100 c.c. with 55 c.c. of methyl alcohol, placed in the viscosimeter cup, acidulated with lactic acid, and readings taken after the successive additions of 0.25 molar NaCl solution. The viscosimeter and dispersions were kept at the temperature indicated in Table VII and Figure 11. The leaching operation and all readings were made at these temperatures. For obvious reasons the procedure did not exactly parallel Sandstedt and Blish's (1933) method, since it was impossible to change the temperature during the examination of each sample. Therefore instead of keeping constant the salt content of the dispersion and varying the temperature, the temperature was kept

TABLE VII

THE INFLUENCE OF ALCOHOL AND OF TEMPERATURE ON THE HYDRATION OF WHEAT FLOUR PROTEIN AS MEASURED BY THE VISCOSIMETER

C.c. of M/4 salt solution added	Acid alone 21°	Acid plus ethyl alcohol 21°	Acid plus methyl alcohol		
			6°	21°	26.7°
		Viscosity			
0	188	220	185	195	196
1	125	146	105	110	122
2	93	115	60	81	90
3	70	92	36	54	67
4	53	74	23	36	51
5	38	55	16	26	35
6	30	46	10	19	28
7	23	35	8	10	20
8	18	30	6	9	16
9	16	26	5	9	14
10	13	22	5	7	13

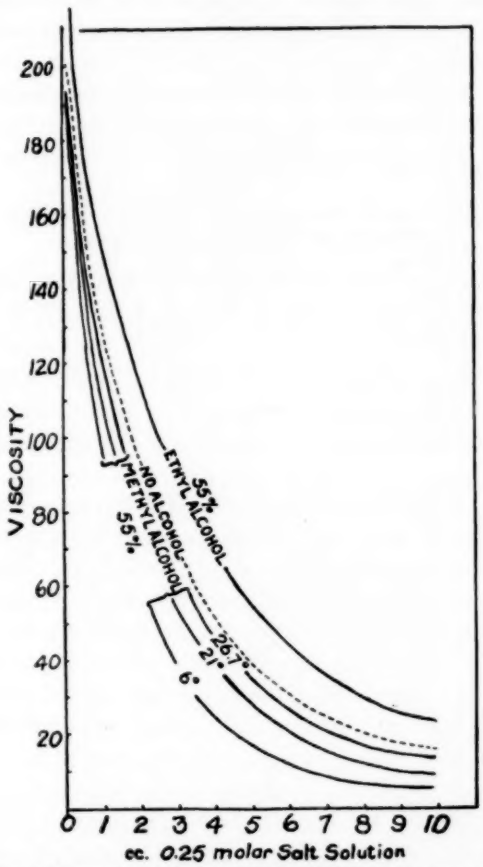

Figure 11. The influence of temperature on the dehydration of wheat flour protein in alcohol-acid dispersions.

constant and the salt concentration was varied. By comparing the several curves the effect of temperature variations at constant salt concentration may readily be seen.

The data and curves show that dehydration occurred at a faster rate, the lower the temperature. Data are also given for the effect of ethyl alcohol as compared with that of methyl. As Sandstedt and Blish's observations might indicate, the curves are at different slopes than those obtained when methyl alcohol was used.

Dill and Alsberg (1925) concluded, from their work, that the dispersion of gliadin in alcohol-water solution is a case of peptization rather than true solution. They found that for a dispersion of gliadin in alcohol of any given concentration there is a fairly well defined temperature at which turbidity develops when the temperature is lowered. When the temperature is raised the dispersion clears at practically the same temperature. They call this temperature the "critical peptization temperature" (C. P. T.). Their data showed that the C. P. T. varied with the alcohol used, and was several degrees higher for methyl than for ethyl alcohol.

The variation on the rate of precipitation of the gluten proteins may possibly be due to the fact that peptizability is related to temperature—higher temperatures favoring peptization.

Relationship between Flour Grade and the Amount of Protein Dispersed by Various Reagents

One of the striking features of the reported work on the peptizability of the wheat flour proteins is the pronounced variability in the amount of protein dispersed by a given salt solution. In searching for an explanation, Gortner, Hoffman, and Sinclair (1928, 1929) came to the conclusion that differences in peptizability probably had their origin in the environmental factors under which the wheat was grown and harvested.

Rich (1933, 1936) has reviewed the literature and as a result of a study of a number of flours of widely different grades and from a study of the data in the literature, showed that when flours of the same grade (*e.g.*, experimentally milled straight grade flours) are studied, the variability between flours is low, while significant variability occurs only in series containing flours of different grades. Correlations as high as + 0.9 were obtained between the amount of protein extracted by a given salt solution and the ash content of the flours. It is altogether probable that the ash constituents themselves are not responsible for the differences in the peptizability of the proteins. It is, however, very probable that the ash content is highly correlated with the *causal factors* determining peptizability. If this is true, then, most of the

differences in the peptizability of the flours are not due to inherent differences caused by environmental or genetical factors, but are due to some factor which varies according to the length of extraction of the flour. In terms of the present system of classification, the amount of non-gluten protein varies with some factor which is associated with the mineral content of the flour.

In this respect some of the data reported by Rich (1936) are interesting. While it was shown that the amount of protein extracted by salt solutions varied directly with the ash content, with distilled water the relation is not as simple. The curve drawn by plotting amount of protein extracted against ash content is apparently rectangularly parabolic, symmetrical about 0.65% ash content. By continuing the curve it might be supposed that *if a flour were ash free* a large proportion of its protein would be extractable by water alone. The data reported by Sharp and Gortner (1923) and Rich (1936) suggest that at least 65% of the total protein could be extracted by water alone from an ash-free flour by a single extraction, leaving the remaining 35% as hydrated particles too large to be decanted or centrifuged from the suspension.

The data reported by Rich (1936) also showed that the amount of protein extracted by a salt solution depends on the concentration of the salt solution—curves plotted from such data rising to a maximum with about 0.25 molar solutions, and then descending as exponential curves. Moreover, as the ash content of the flours becomes less, the rising part of the curves becomes less, until for a flour of about 0.39% ash content, the amounts extracted by 0.25 molar NaCl solution and distilled water are equal. With flours of less ash content than 0.39% there is no upward curve.

In Figure 12 curves for a series of 12 flours, varying in ash content from 0.04% to 1.14%, have been drawn, in which the molality of NaCl solution is plotted against the amount of "soluble" protein. Each flour differs in ash content by 0.10%. In the range where actual flours were studied the actual data were used, but the series was extended on both sides of this range, using interpolated values obtained from the curves in Figure 1 of the previous study (Rich 1936).

The curves for the low-ash flours are interesting. Whereas a 10% NaCl solution extracts appreciable amounts of protein from actual flours, an ash-free flour would apparently contain almost no protein "soluble" in such a solution. Looking at it from another viewpoint, a 10% NaCl solution would actually salt out nearly all the protein which had already been dispersed by the water of the suspension.

The curves suggest that the ash constituents are in some way related to the ease with which the proteins can be extracted by neutral solvents (*i.e.*, by ions other than the H^+ or OH^- ions). It is difficult to conceive,

if they are considered simply as salts occurring in the flour and free from the protein, how such relatively small amounts could have any measurable effect on the peptizability.

Working (1924, 1928), Geddes (1930) and others have given some evidence that the phosphatides present in the flour have an important influence on protein properties. Blish (1936) reported that his labora-

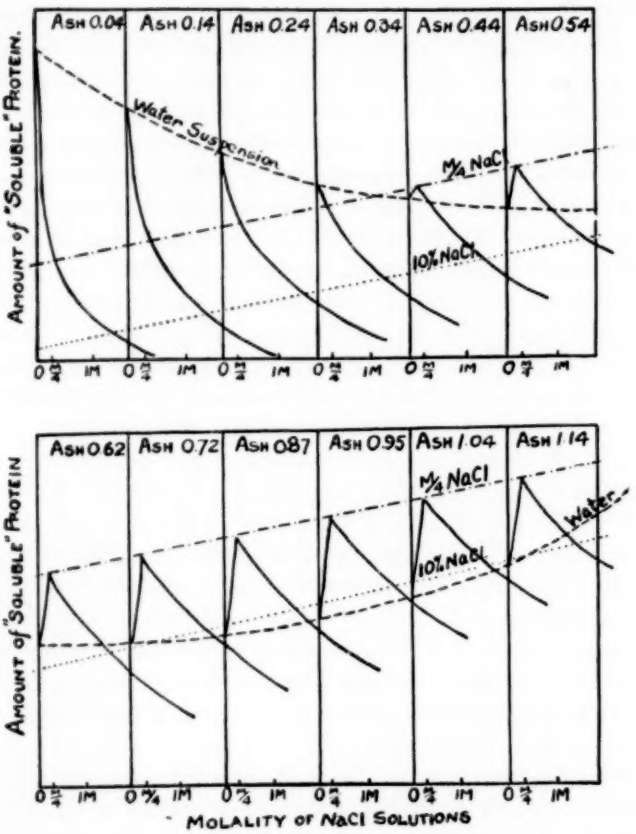

Figure 12. Schematic representation of the "solubility" of wheat flour protein in 12 flours of varying ash contents in the presence of salt solutions of varying concentrations.

tory had some evidence of a "lecitho-protein" or some other lipoid-protein complex being present in wheat flour gluten. The curves in Figure 12 may possibly be interpreted by the assumption of this lipoid-protein complex, since in lecithin and related substances phosphorous is a part of the molecule. In this manner *part* of the ash, at least, would be linked with the protein, and the "solubility" of the protein in flours of different ash contents may possibly be due to the

presence of varying proportions of this complex. The "washing" or leaching of flour suspensions with distilled water, then, probably not only leaches the soluble salts from the flour but partly disrupts this complex as well.

Further evidence of the existence of this complex is that if the protein be completely dispersed in dilute acid solution (at pH = 3) or in sodium salicylate solution, it can be re-precipitated without the protein suffering any profound change. On the other hand, if the protein is extracted, or even suspended in alcohol and the alcohol evaporated spontaneously without extracting anything, a decided change takes place, and the protein no longer possesses its original properties. This phenomenon may be due to the disruption of the protein-lipoid complex by the alcohol.

Not enough data have been collected as yet on which to base definite conclusions, and further study is needed to elucidate this problem.

General Discussion and Conclusions

It is evident that in fractionating and isolating portions of the total protein material of wheat flour we are dealing with the characteristics of a colloidal system, and that many of the "fractions" obtained by selective "solution" and precipitation are simply the results of certain reagents on a colloidal system. Solution, in so far as the protein material of wheat flour is concerned, is in reality peptization. Aside from such factors as temperature, ratio of flour to "solvent," *etc.*, the proportion of the total protein dispersed by aqueous solutions of various reagents is dependent on the nature of the ions and on their concentration in the suspension. The ions can be arranged in the order of the Hofmeister series according to their dispersing power. A partial series for the anions is as follows:

$$F < SO_4 < Cl < Br < I < CNS < \cdots < \text{salicylate} < OH$$

while the cations may be arranged (incompletely) as:

$$Na < K < Li < Ba < Sr < Mg < Ca < \cdots < H$$

For neutral salts (where the H^+ or the OH^- ions do not play a part) maximum dispersion seems to occur with solutions approximately 0.25 molar. This is not a constant value because solutions of salts containing bi- or tri-valent ions disperse the greatest amount of protein at concentrations somewhat less than 0.25 molar.

The data of Sharp and Gortner (1923) are interesting from this standpoint. By adding successive increments of acid solutions to flour-in-water suspensions, they noted first a rapid increase in viscosity,

caused by the hydration of the protein, followed by a decrease. With the exception of the curves for lactic and acetic acids the curves were similar in form and resemble those obtained by plotting "dispersed protein" against salt concentration. Because the H ion was the chief factor influencing the hydration (and dispersion) of the protein, Sharp and Gortner (1923) restated their data in terms of H-ion concentration (pH). When viscosity was plotted against actual pH values the curves became regular and were similar to the idealized form suggested by Gortner (1929) and reproduced in Figure 13 of this paper. The reason for the apparent difference in the behavior of lactic and acetic acids then became obvious.

Referring to the action of salt solutions on protein, it is wholly probable that it it were possible to state the actual effective concentration of other ions than H or OH ions, then the data obtained by using

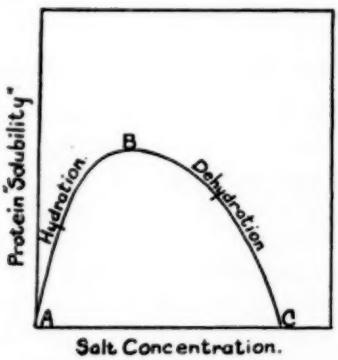

Figure 13. A diagrammatic representation of the peptization process.

salt solutions as the dispersing agents would be represented graphically as shown in Figure 14.

While Sharp and Gortner's curves indicate that the H or the OH ion, as the case might be, is the chief factor influencing the hydration of the protein, it by no means governs it entirely. The relative effects of the anions is also clearly shown by the height to which the curves ascend, and a distinct Hofmeister series is apparent in their data, the SO_4 ions having the greatest depressing effect and the PO_4 ions the least. Anion effect is also shown in the data presented by Gortner, Hoffman, and Sinclair (1929) concerning the peptizing power of various salt solutions.

Whether a given salt will salt-out a given dispersed protein depends on whether sufficient dehydration of the protein can be reached before the solubility of the salt in water can be reached. Speaking generally, this depends on the height and slope of the curve produced by plotting

the amount of dispersed protein against ion concentration, and is dependent on both the nature of the ions composing the salting-out reagent and the nature of the protein in question. While the "salt-soluble" proteins of animal origin can be salted out by such salts as Na_2SO_4, $MgSO_4$, *etc.*, this is not true of many of the vegetable proteins (including wheat-flour protein). This is indicated by the curves obtained for the latter proteins, which do not reach the abscissa of the graph before the solubility of these salts is reached (see Rich (1936) Figure 2).

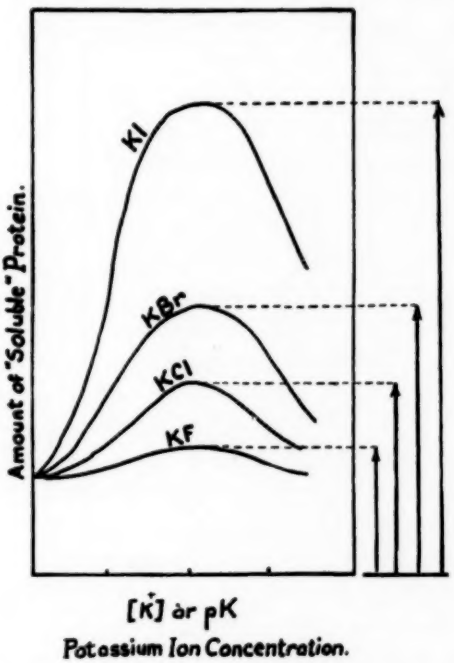

Figure 14. Schematic representation of the "solubility" of wheat flour proteins in different salt solutions, in which one of the ions of the salt is plotted in terms of "effective concentration."

One of the disadvantages involved in the isolation of the protein from flour is that maximum dispersion occurs at maximum hydration, or rather that the degree of hydration is so great. In the case of the "fraction" most difficult to disperse, the fully hydrated particles are so large that they can not be separated from the starch of the flour-water suspension. Blish (1936) stated that about 10 to 15% of the total protein is in such large particles when dispersed in dilute acetic acid or in sodium salicylate solution that they can be thrown out by the supercentrifuge. McCalla and Rose (1935) found that the first fraction salted out by $MgSO_4$ from dispersions in 12% sodium salicylate solution was still a hydrated mass, and that the precipitated substance

became less gummy as the fractionation proceeded. The last fraction was a fine, powdery substance. This may be taken as evidence that the dispersed fractions vary in size. Krecji and Svedberg (1935) also gave same evidence that the fraction which is dispersed by alcohol exists as particles of different sizes.

Another problem encountered in the study of the protein of wheat flour by dispersing it in the flour-in-water suspension is that those ions which have the greatest peptizing or dispersing effect on the proteins behave in a similar manner towards the starch. Separation of the

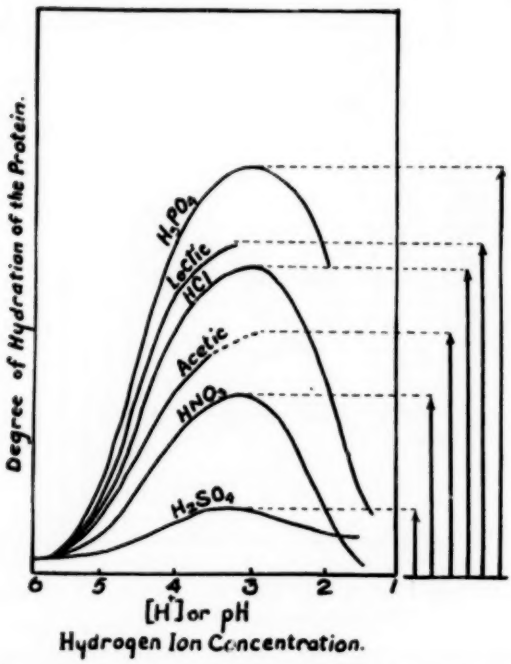

Figure 15. The influence of hydrogen ion concentration on the hydration of wheat flour protein, and the hydrating power of different anions. Taken from the data of Sharp and Gortner (1923).

hydrated protein from a suspension of swollen starch grains is difficult if not impossible. To eliminate this difficulty by using crude gluten as a starting point is not wholly satisfactory, since it is known that the gluten washing process is decidedly an arbitrary one, and the gluten may vary both in chemical as well as physical properties by varying any one of a number of factors involved in the washing process. Moreover, the "water-soluble" and part of the "salt-soluble" proteins are lost before the experiment begins.

The possibility of a lipoid-protein complex as suggested by Blish (1936) is indicated by the relationship between the amount of protein

extracted by various neutral solvents and the ash content of the flour. The action of alcohol on the proteins would also suggest this possibility. Not enough data have been obtained as yet to warrant a definite conclusion, but apparently part of the ash constituents are linked to the protein in a complex of this nature.

Alcohol, although it is a well known dehydrating agent, apparently has no effect on the hydrated particles which have been designated the "glutenin" fraction, possibly because they are so highly hydrated that the presence of alcohol solution is not sufficient to bring the charged and hydrated particle to below its critical hydration point.

A great deal of work has been done in past years in analyzing the so-called individual proteins of wheat flour (and many other proteins) by the van Slyke and other methods. The most striking feature of such work is the lack of agreement between investigators, of the data, and even of different analyses of the same type of material by the same author. The reason for this is easily apparent. Wheat-flour protein, when dispersed, is a lyophilic colloidal substance, and hence is so labile that unless extreme precautions are taken in the method and technique of isolation, no two fractions will be identical in either amount or in chemical composition. It would appear that until the colloidal properties of wheat-flour protein are better understood, analyses of arbitrary fractions will be of little value.

Whether the total protein of wheat flour exists as several distinct individuals, or whether it is a single complex, and that certain ions peptize fractions of varying size and composition from the main particle, is not yet clear. The evidence presented in this paper suggests the latter probability, although it is not yet certain that similar data could not be obtained from a mixture of several individuals.

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Correction

In former papers, Cereal Chem. **10**: 222–232 (1933) and in Cereal Chem. **13**: 522–541 (1936), the experimental work was done with *molar* instead of *normal* solutions, as was wrongly stated.

C. E. R.

THE PREPARATION AND PROPERTIES OF WHEAT PROTEINASE [1]

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(Read at the Annual Meeting, May 1938)

Since the present authors succeeded in identifying the proteinase of wheat as belonging to the papain type of enzymes (1935), considerable work has been done on the subject and a number of decidedly contradictory conclusions have been published. The matter is of importance because of the softening effect that proteinase has on the dough. If the properties ordinarily ascribed to papain are admitted as applying to the wheat enzyme, the conclusion is self-evident that oxidizing agents will inactivate the enzyme and therefore, with appropriate flour, will act as bread improvers. The improvement of bread attendant on the storage of flour in air, the bleaching with oxidants, or the addition of bromate, persulphate, and the like directly to the dough is then readily explained. The extremely small amount of oxidant required is not surprising because its action is not on the main constituents of the system but on a catalyst, itself present only in traces. The action of all oxidative bread improvers would thus be fundamentally understood.

All of this was suggested previously by the present authors and for practical purposes coincidentally by Jørgensen (1936). Subsequently Flohil (1936) redetermined the experimental data and reached confirmatory conclusions. However, later investigators, particularly Reed and Haas (1937) seem to have had difficulty in reconciling their conception of wheat proteinase with these findings. Reed and Haas state relative to their data, that they "did not provide any basis for concluding that bromate inhibits the action of the proteinase of wheat flour." In thus reaching conclusions diametrically opposite to ours, these authors have reopened the whole question of the similarity of the wheat enzyme to papain and the action of oxidative bread improvers.

We have therefore made a second study of the proteinase in wheat, taking advantage of newer and more sensitive methods that have been developed in the meantime for the demonstration of proteolysis. Starting with bran, which we previously found to contain relatively

[1] Food Research Division Contribution No. 383.

high amounts of wheat proteinase, we have been able to remove the enzyme from the raw material and thereafter to concentrate and purify it. Our best preparations have shown a proteolytic activity of about the order of one-four-hundredth that of crystalline papain, in other words an enzymic activity of no small magnitude and easily demonstrable by the usual methods. We are thus in a position to study the characteristics of wheat proteinase without fear of disturbance from the great quantities of admixed material that occur along with it in nature, and from the errors that attend the use of greatly diluted enzymes.

Purification of the Wheat Proteinase

The proteolytic enzyme was extracted at 0° from finely ground bran by dilute ammonium sulphate containing a trace of cysteine for the protection of the enzyme. The enzyme was then concentrated by fractional precipitation with ammonium sulphate. The protein fraction precipitated between 0.4 and 0.8 saturation contained the enzyme. It was dried in the cold and constitutes a fairly stable crude material for further purification work. Re-solution of the dried filter-cake in water and dialysis in the cold against 25% glycerin (containing a trace of cysteine) caused a heavy precipitation of globulin. This was removed by centrifuging in the cold. The resulting solution of the enzyme in dilute glycerin was made more stable by bringing the glycerin concentration up to 50%. This preparation was found to be quite satisfactory for study. Purification is also possible through fractional precipitation with acetone, and in other ways which will be reported later. The enzyme was completely precipitated between 50 and 75% acetone.

The determination of enzyme activity throughout the process just described was made by a viscosity method with gelatin at pH 5. The method is essentially that described by Northrop (1932) for proteinases. The results are reported in terms of milligrams of a dry preparation which was obtained by the acetone method early in our work and adopted as a basis for reference. Because the drop in the viscosity of gelatin is a rather complicated function of the time and the enzyme quantity, a set of empirical curves was prepared with the reference preparation, using various amounts of enzyme and measuring the viscosity at arbitrarily chosen times, namely 15, 30, 45, and 60 minutes. By reference to these curves (Figure 1) the value of an unknown preparation can be expressed in milligrams of the reference preparation used to make the curve. Subsequent experience has given us good reason to rely on these results. The variations observed among dupli-

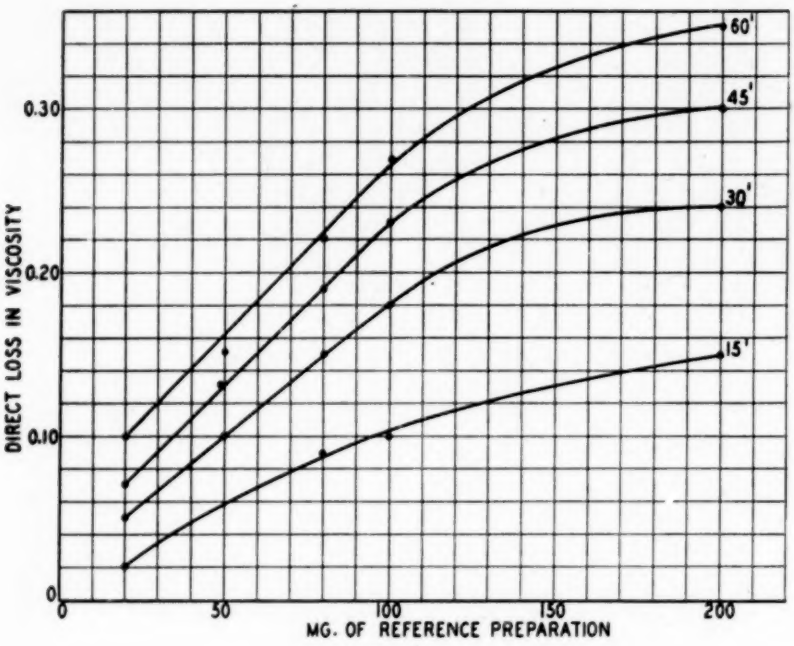

Figure 1.

cate and multiple quantities of enzyme on numerous occasions were less than 10% and frequently less than 5%.

The process of purification, as shown by the method just described, is illustrated in Table I. The values refer to milligrams of the (dry) preparation originally selected for reference. The experimental set-up here and in all cases, unless otherwise stated, consisted of 5.0 c.c. of

TABLE I

PURIFICATION OF WHEAT PROTEINASE (ACTIVATED WITH CYSTEINE)

	Process of purification	Activity per mg. of protein N	Percentage recovery
A	Extract from bran	—	Taken as 100
B	Precipitate from A between 0.4 and 0.8 saturation with ammonium sulphate, dried	23	100
C	B dissolved in water, dialyzed against dilute glycerin, globulin removed, glycerin added	45	70
C′	B dissolved in water, acetone added to 75%, precipitate dried	39	55
C″	B dissolved in water, acetone added. Precipitate collected between 50 and 66% acetone	77	35

water containing whatever enzyme or activating or inhibiting substance was used. The activator was usually 10 mgs. of neutralized cysteine hydrochloride. The enzyme etc. was added to 5.0 c.c. of 6% gelatin at a pH of 5 and containing M/20 citrate. The temperature was 35.5°.

Activation of the Wheat Proteinase

Solutions of the wheat proteinase at any stage of purity so far reached were unstable. Relative stability was conferred by the presence of salts and particularly of glycerin. Gradual deterioration of proteolytic activity still occurred, however, accompanied with a darkening of the solution that suggested the complicity of a phenol oxidizing enzyme. None of our preparations showed marked activity unless treated with cysteine or a similar activator prior to testing, even though cysteine had been used in the previous extraction of the enzyme. Totally inactive enzyme could also be completely restored to its original potency by the addition of cysteine.

The activating effect of cysteine is clearly shown by these data. Cyanide and hydrogen sulphide were observed to have similar activating effects (Table II).

TABLE II

ACTIVATION OF WHEAT PROTEINASE BY CYSTEINE, CYANIDE, AND HYDROGEN SULPHIDE

Additions to enzyme	Time of activation	Reference enzyme	Activity per mg. of protein N
	Min.	*Mgs.*	
None	30	0.00	0
2 mgs. cysteine hydrochloride (neutralized)	30	12	6
4 mgs. cysteine hydrochloride (neutralized)	30	70	37
10 mgs. cysteine hydrochloride (neutralized)	30	110	58
KCN 2 drops 2M. neutralized	10	25	13
KCN 2 drops 2M. not neutralized	3	45	24
H_2S 1 c.c. saturated water solution	30	50	26

Note: For each experiment 2 c.c. of glycerin preparation (Table I) was used. This enzyme had stood for six weeks in air at 5° after preparation.

The proteolytic activity of this enzyme in the presence of cysteine was demonstrated by other methods than that based on the reduction

of the viscosity of gelatin. The enzyme clots milk (Balls and Hoover, 1937) and also digests casein as determined by the well-known alcoholic titration method (Balls, Swenson, and Stuart, 1935) (Table III). In view of the large titration values obtained with casein for digestions lasting a long time (20 hours), there is reason to suppose the activity of the proteinase to be supplemented by that of an accompanying peptidase. On the other hand the viscosity measurements that gave striking results in intervals as short as 15 minutes seem to be referable to proteinase rather than peptidase action.

TABLE III

MILK CLOTTING AND CASEIN DIGESTION BY WHEAT PROTEINASE AT 40° C.

Milk clotting		Casein digestion			
Enzyme	Time of clotting	Amount of enzyme	Set-up	N/10 KOH after 2 hrs.	N/10 KOH after 20 hrs.
c.c. 1	*Min.* 30	*c.c.* None	Casein alone	*c.c.* 0.00	*c.c.* 0.00
2	18	2	No casein	0.00	0.00
2	17.5	2	Casein + 10 mgs. cysteine	0.55	2.10
3	14	5	Casein + 10 mgs. cysteine	1.10	4.05

Inhibition of the Wheat Proteinase

In view of the activating effect of reducing agents such as cysteine on the wheat proteinase it is not surprising to find that oxidizing agents are inhibitory. The harmful effect of storing the enzyme in air has already been discussed. Very small amounts of several common bread improvers, persulphate, bromate, and metavanadate, also destroyed enzymic activity. If the enzyme had been previously activated by cysteine, such activity disappeared; if the unactivated enzyme was treated, later addition of cysteine no longer produced an activating effect. The enzyme behaved as though it had been oxidized to a stage where subsequent reduction was unable to reproduce the original molecule. These data are presented in Table IV.

The inhibition of papain by iodoacetic acid is an important characteristic of the enzyme. This distinguishes papain from cathepsin (Anson, 1937), which is not activated by sulphydryl, but may be accompanied by a peptidase that is. Thus if iodoacetic acid fails to inactivate, or if inhibition is evident only after a digestion period so

long that peptidase action could be an important factor in the result, the presence of a cathepsin accompanied by a sulphydryl-activated peptidase might be considered. This, however, did not prove to be the case, for the enzymic activity was quickly and almost completely lost in the presence of 0.01 molar iodoacetic acid. This experiment confirms the observation that the wheat proteinase is activable by sulphydryl and therefore an enzyme of the papain type. On the other hand nothing can be said with certainty as yet about the properties of the peptidase, if any, that appears to accompany the proteinase as far as we have carried the purification.

TABLE IV

INHIBITION OF WHEAT PROTEINASE BY PERSULPHATE, BROMATE, METAVANADATE AND IODOACETIC ACID

Mgs. of reference enzyme found after 30 minutes' exposure to the inhibitor

C.c. of enzyme + 4 mgs. cysteine	None	Persulphate		Bromate	Metavanadate		Iodoacetic acid M/100[1]
		1 mg.	2 mgs.	1 mg.	1 mg.	2 mgs.	
2	70	25	0	0	25	0	20

[1] Exposure for 10 minutes.

Summary

The proteinase of wheat, perhaps still accompanied by a peptidase, has been separated from bran by chemical means and considerably concentrated. The properties of the partially purified proteinase show that it is an enzyme of the papain type. It becomes inactive on standing in air and may be reactivated by the addition of cysteine. The active enzyme digests casein, clots milk, and lowers the viscosity of gelatin. It is also inactivated by several oxidative bread improvers, namely persulphate, bromate, and metavanadate. Further confirmation of the papain-like character of the wheat proteinase is given by its inactivation with iodoacetic acid.

Since it may be fairly concluded that the proteinase of wheat is an enzyme similar to papain, its inactivation by oxidizing agents such as the usual bread improvers still seems to explain adequately the effect of the latter in the dough.

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THE CEREAL AMYLASES WITH REFERENCE TO FLOUR AND MALT BEHAVIOR [1]

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(Read at the Annual Meeting, May 1938)

Within recent years there have appeared from this laboratory several published reports dealing, respectively, with various aspects of wheat amylase activity. The reports have been concerned with studies of identities, properties, and quantitative measurements of cereal amylases, and with related matters, including the factor of starch susceptibility to amylase action.

The main objective in these studies has been to establish a rational explanation of diastatic phenomena associated with dough fermentation, thereby affording advantages in the technological control of flour and bread production. As the work has progressed, however, it has become apparent that certain developments might also find useful application elsewhere, as for instance in malting and brewing technology.

The purpose of this paper is to assemble the findings and conclusions derived from previous as well as later and unpublished data, and to correlate and reconcile these findings with modern beliefs of other workers, in an effort to present as completely as prevailing circumstances will permit the essential features of amylase activity and properties with special reference to flour behavior and cereal technology.

The present state of our knowledge and beliefs regarding the fundamentally important aspects of amylase identity and amylase behavior in relation to starch structure and properties has recently been extensively discussed in an admirable review by Hanes (1937).

General Considerations Relative to Cereal Amylases

There is as yet no basis for general agreement or understanding as to the number and identities of all of the individual biocatalysts, respectively, that may participate in the initiation and control of diastatic processes. Justification for the recognition of at least two distinct factors seems established beyond reasonable doubt. Beta-amylase, the so-called "saccharogenic" amylase, is the predominating

[1] Published with the approval of the Director as Paper No. 212, Journal Series, Nebraska Agricultural Experiment Station.

type occurring in sound, ungerminated grain. Germination or malting of the grain presumably induces the production of a second type, called alpha-amylase, the "dextrinogenic" amylase. Some authorities consider that the alpha form exists also in ungerminated cereals, although largely in a latent or inactive state, but this is a controversial issue.

Using viscosimetric technique, the liquefaction of starch paste, as distinguished from saccharification, has been investigated by a number of workers, including Gore and Jósza (1932), Jósza and Gore (1932), Jósza and Johnston (1935), and Waldschmidt-Leitz and Mayer (1935). Jósza and Johnston (1935) consider the "liquefying" enzyme to be identical with alpha-amylase, while Waldschmidt-Leitz and Mayer (1935) regard it as a distinct factor whose chief function is to split off esterified starch phosphorus, thereby permitting full reactivity of terminal aldehyde groups. They have designated it as "amylophosphatase." Assuredly this phase of amylase activity needs further study.

Regardless of the degree of certainty with which the properties of these enzymes have been identified and characterized, our knowledge of them is based entirely on observations of their activities upon *soluble* or *gelatinized* starch. Comparatively little study has been devoted to the amylolytic digestion of the native or "raw" starch as it occurs in sound, mature grain. It seems to be rather commonly supposed that the raw starch is rendered susceptible to diastasis by the action of cytolytic enzymes which destroy the cellulose-like envelope supposedly surrounding the starch particle, thus exposing the readily soluble and easily hydrolyzable inner portion.

It is conceivable that the enzymic saccharification of *raw*, native wheat starch might be an important factor of sugar production during the autolytic digestion of flour in dough fermentation. Blish, Sandstedt, and Mecham (1937) have shown that apparently all flours contain appreciable though variable amounts of an enzyme factor capable of catalyzing the saccharification of raw wheat starch. Germinated grains, or malt, contain much greater amounts, as would of course be expected. The occurrence of this factor parallels that of alpha-amylase in that both are low in sound, mature grain and abundant in germinated material, which is not at all surprising. In some properties, however, there seems to be enough lack of parallelism between the two factors to justify the presumption that the raw starch factor is a distinct enzyme apart from those already recognized.

Should the raw starch catalyst be classified as an amylase or more properly as a cytase (hemicellulase)? As will be shown later, the nature of its rate curve, when acting on raw wheat starch, is more

suggestive of amylolytic than of cytolytic action. Possibly the term "cyto-amylase," or "amylocytase" might be an acceptable designation.

It is quite probable that, depending upon conditions, amylase activity may be strongly influenced by certain activators, kinases, inhibitors, etc., and several such biocatalysts have been reported from time to time. There is, for example, the so-called "amylokinase" reported by Waldschmidt-Leitz and Purr (1932), but whose occurrence other workers have not been able to confirm. Failures of one worker to substantiate the findings of another in these matters has thrown the whole situation into such a state of uncertainty that no serious consideration of this aspect of amylase activity will be attempted in this discussion.

Chemical nature of amylases.—The fact that the amylases are completely precipitated from a grain or malt extract by high concentrations of ammonium sulfate, as demonstrated by Osborne (1895) more than 40 years ago, suggested that they are proteins or in any event closely associated with them. That flour amylase is more rapidly and completely extracted by neutral salt solutions than by water alone was shown by Jósza and Gore (1932), and by Sandstedt, Blish, Mecham, and Bode (1937). This offers the possibility that the amylase is most highly concentrated in the so-called "globulin" fraction. The colloidal nature of amylases is indicated by their failure to pass through a dialyzing membrane.

There is however much circumstantial evidence favoring the viewpoint that amylases need not be regarded as proteins, but that they are merely associated with or bound by the latter, which serve chiefly as "carriers." It is known that under certain conditions amylase activity can be liberated by the destructive action of proteolytic enzymes, especially papain, as shown for example by Chrzaszcz and Janicki (1936) and by Myrbäck and Örtenblad (1937) who find that ungerminated grain contains considerable though varying amounts of "latent" amylase that can be set free by treatment with papain, or with H_2S, which is known to be a proteinase activator. If indeed the amylases are true proteins one might expect them to undergo rapid destruction by the strong proteolytic activity manifested during germination, but obviously what actually happens in the malting process is an increased rather than a reduced amylase activity.

Extraction, concentration, purification, etc., of amylases.—As already stated, wheat-flour amylase is more readily extracted by salt solution than by water alone. Sugars and other soluble substances can be removed by direct dialysis, or by precipitating the amylase with alcohol or with ammonium sulfate. High temperatures with high

alcoholic concentrations have a destructive effect (Caldwell and Doebbling, 1935, and Blish, Sandstedt, and Mecham, 1937). Dialysis of ammonium sulfate precipitates is needed to remove salt and cause redispersion of most of the amylase. These dispersions, saturated with toluol, will keep for a long time without serious deterioration. They may be concentrated further by pervaporation if desired.

In comparative studies of starch saccharifying powers of various preparations, it is of course essential that both temperature and pH be controlled. A suitable pH may be found in the range 4.5–5.0, the acetate buffer (pH 4.7) recommended by Blish and Sandstedt (1933) being satisfactory and convenient. The ferricyanide method of Blish and Sandstedt (1933) is a simple and satisfactory procedure for the estimation of reducing sugars (calculated as maltose) at progressive time intervals.

Beta-Amylase

Beta-amylase is the diastatic enzyme characteristically predominant in ungerminated cereals. It occurs abundantly in wheat flour, in ungerminated barley, and doubtless in other cereals. Some workers have stated a belief that beta-amylase is the only one present in ungerminated grain, but this seemingly is not strictly true. Blish, Sandstedt, and Mecham (1937) found small, variable, but measurable quantities of other amylases (alpha-amylase and the "raw starch" saccharifying factors) in all of the wheat flours that they examined, but the amounts were small in comparison with the overwhelmingly large quantities of beta-amylase.

Beta-amylase is distinguished by its ability to saccharify starch that has been rendered susceptible or "soluble" by treatment with acid, by gelatinization in hot water, by mechanical disintegration, or by a combination of these treatments. It is practically without ability to saccharify raw, untreated starch. According to the best evidence, as summarized by Hanes (1937), "This amylase induces an end-wise type of degradation, in which successive terminal disaccharide fragments are split off from one end of the molecular chain structure of the substrate." Quoting again from Hanes (1937) "the hydrolysis comes to an end when approximately 60% of the starch substance has been converted to maltose . . . the reaction proceeds relatively rapidly until the reducing power reaches a value corresponding to 50–55 per cent of the theoretical maltose; thereafter greater resistance is evident and the reducing value rises only slowly to its final level." At the completion of the reaction there remains a residual dextrin, representing nearly 40% of the original starch, called alpha-amylodextrin or "erythrogranulose." It is colored blue with iodine,

and is precipitable with alcohol. It is quite resistant to any further action of beta-amylase, but not to alpha-amylase, as will be shown later.

Differences in beta-amylase concentration are shown by corresponding differences in the rates at which the 60% starch conversion point is reached, and this provides the basis upon which quantitative comparisons can be made.

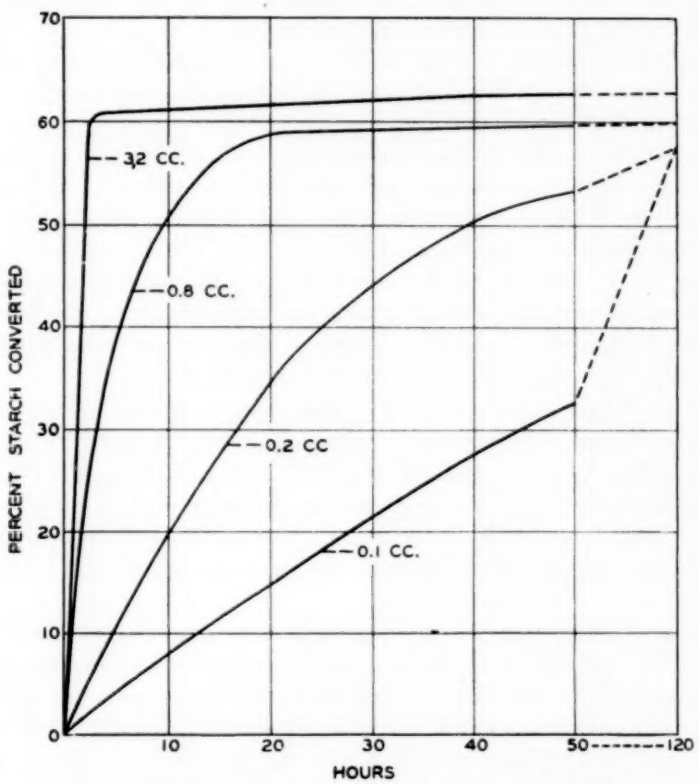

Fig. 1. Influence of enzyme concentration on beta-amylase activity.

The data in Figure 1 show in a typical manner what occurs when varying quantities of the same beta-amylase preparation are allowed to act upon soluble starch under strictly comparable conditions. A dilute extract of soft wheat flour was acidulated to pH 3.3 (in an ice bath), in accordance with Ohlsson's (1922) directions for inactivating any appreciable quantity of alpha-amylase that might be present. After neutralization with $NaHCO_3$, the amylase was precipitated with 40% $(NH_4)_2SO_4$ and the precipitate dialyzed for two days. To four separate 100-c.c. portions of 1% soluble starch (boiled potato

starch) were added, respectively, 0.1, 0.2, 0.8, and 3.2 c.c. of the dialyzed and diluted enzyme solution. Rates of saccharification were ascertained by maltose determinations (ferricyanide method) on small aliquots withdrawn at successive time intervals.

The rate curves in Figure 1 show that all of the enzyme concentrations achieved approximately the same *degree* of saccharification (60% starch conversion), but the *rates* varied greatly. This emphasizes the fact that *rate* rather than *degree* of saccharogenesis is the important consideration in quantitative measurements.

In another series of experiments beta-amylase preparations were made from soft wheat flour and from ground whole barley, using for extraction one part of dry material to five parts of water. The extracts were acidified to inactivate any alpha-amylase present. After neutralization the amylase was precipitated with $(NH_4)_2SO_4$, followed by dialysis of the precipitates, the dialized amylase solutions being adjusted so that 1 c.c. was equivalent to 0.7 gm. of original grain. Both with the wheat and barley amylases, 0.2, 0.8, and 3.2 c.c. portions were allowed to act respectively on 100 c.c. portions of 1% boiled starch as in the previous experiment. The rates and degrees of maltose production are shown in Figure 2.

From the data in Figure 2 it is seen that, as before, approximately the same *degree* of starch conversion (60%) was reached in all cases. Beta-amylase activity was far greater in the soft-wheat flour than in the whole-barley extract, as shown by the respective saccharification rates. This however may have been due to the fact that water extracted the amylase more completely from the wheat than from the barley.

A highly important feature of the data in Figure 2 appears from the fact that, in contrast to the barley, the *wheat flour* amylase was so extremely active that the conditions of the experiment fail to reflect any useful quantitative differences between the highest and the lowest concentrations. The rate curve for the 0.2 c.c. portion is almost identical with that for the 3.2 c.c. portion, which contained 16 times as much amylase. This strikingly emphasizes the need for establishing suitable conditions if quantitative distinctions are desired. In this instance either the amounts of amylase should have been greatly reduced by dilution, or the time intervals should have been chosen in minutes rather than hours. In the case of the barley extracts, on the other hand, the conditions afford a good basis for establishing quantitative differences.

The foregoing experiments and considerations make it easy to understand why the Lintner type of method provides no useful means for differentiating flours as to their diastatic properties. Being

abundantly supplied with beta-amylase, all flours saccharify Lintner or other forms of soluble starch very rapidly. But flour starch is certainly not Lintner starch, and it has long been recognized that useful and informative differentiation is possible only when the autolytic or Rumsey type of method is employed, whereby the substrate is the flour's *own* starch, instead of an artificially prepared, readily available substrate. It appears certain that the wide variations actually found in the autolytic saccharifiying powers of flours—in view of their uni-

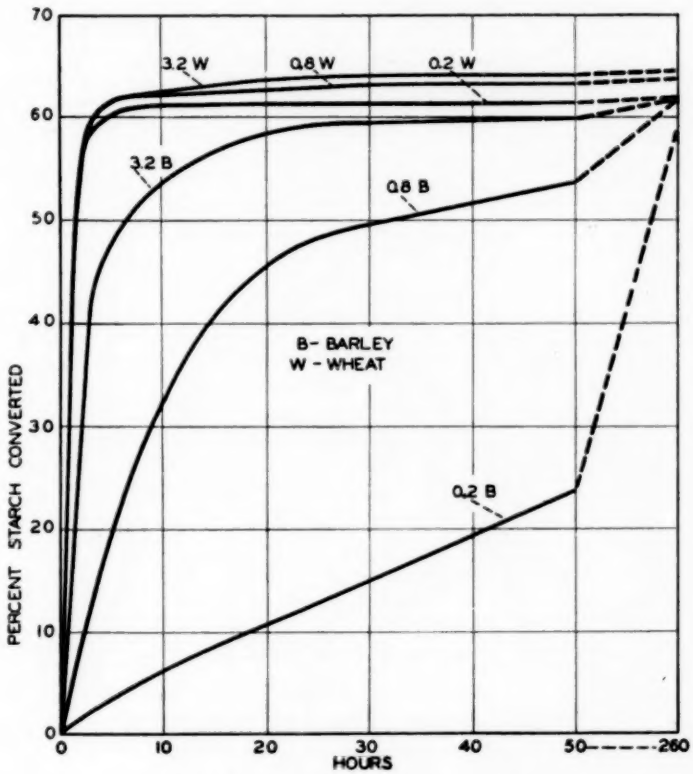

Fig. 2. Beta-amylases from wheat and barley.

formly high beta-amylase content—are due to differences in amount or nature of the substrate, and not to variations in quantity of beta-amylase. However, alpha-amylase, if present, can be an influencing factor, as will appear later.

Autolytic diastasis in flour.—The rate curve for autolytic diastasis in a normal wheat flour usually shows characteristics strongly suggestive of beta-amylase acting on soluble starch (Figures 1 and 2). The chief point of resemblance lies in the fact that saccharogenesis proceeds

very rapidly at first, but after an hour or slightly more it diminishes to a very slow rate. There are several reasons for the supposition that this sharp falling off in rate is due almost entirely to exhaustion of the supply of available starch substrate, and not to any "using up" or exhaustion of the active enzyme:

(1) It can be shown that an abundance of active beta-amylase is present even after activity has almost ceased entirely. If some boiled starch solution is added to the digestion mixture, an active and rapid saccharogenesis is at once resumed.

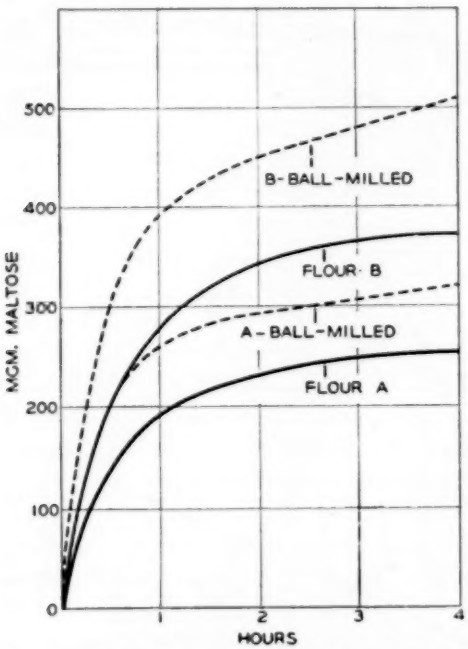

Fig. 3. Effect of ball-mill grinding on flour-starch susceptibility.

(2) That only a small portion of the *total* flour starch is saccharified by the beta-amylase is obvious from a consideration of the quantity of maltose ordinarily produced. If however the flour is subjected to additional grinding, as in a ball mill, the amount of autolytic saccharogenesis is substantially increased.

(3) Raw, untreated wheat starch is to all intents and purposes immune to diastatic attack by beta-amylase. In one experiment an active beta-amylase preparation saccharified only 1% of raw wheat starch in 500 hours, and this almost negligible action was probably due to the presence of a trace of another enzyme.

(4) Commercially milled flours show higher diastatic powers than flours milled on a small experimental mill from the same wheats, respectively, due in large part to a more severe grinding of starch particles in the commercial process.

Figure 3 shows typical autolytic rate curves for two normal baker's flours, both before and after grinding in a ball mill. Figure 4 presents the rate curves for a commercial flour as compared with flour milled experimentally from the same lot of wheat.

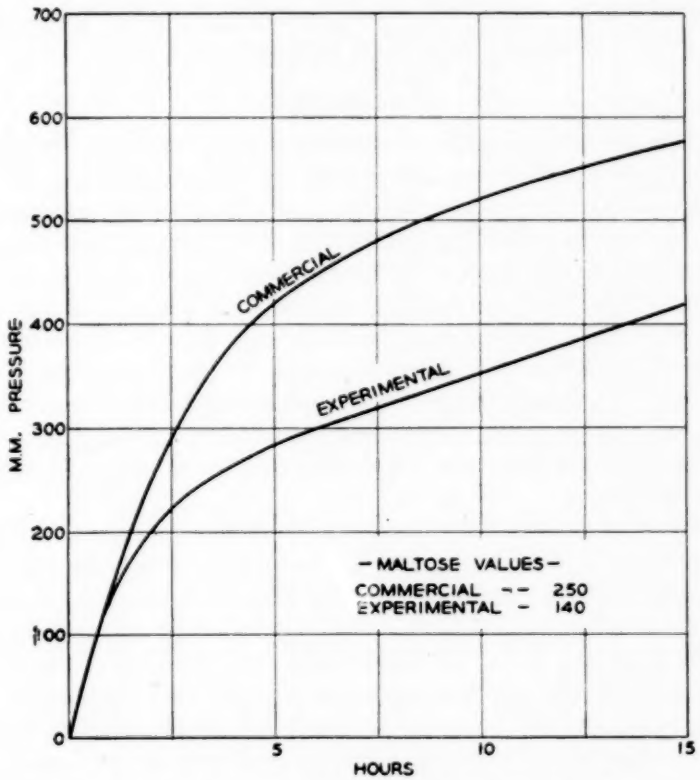

Fig. 4. Autolysis curves for experimentally and commercially milled flours.

It is apparent from Figures 3 and 4 that a portion of the "susceptible" (to beta-amylase) starch in flour is the result of the mechanical rupture of raw starch during milling.[2] It seems likely, however, that at least a significant amount occurs naturally in the form of dextrins or closely related substances.

Summing up this phase of the discussion the autolytic diastasis

[2] The extremely high maltose values of durum flours appear to be due to extraordinarily large amounts of "susceptible" starch (Sandstedt, Blish, Mecham, and Bode, 1937).

of normal wheat flour can be explained as follows: Beta-amylase, characterized by its ability to saccharify about 60% of "soluble" starch, is the principal amylase occurring abundantly in ungerminated grain, and in wheat flour. It is inactive on raw starch, which constitutes the greater portion of flour. However, flours contain small, variable amounts of a "susceptible" starch, comparable to "soluble" or Lintner starch, and the quantity of this is somewhat dependent on the degree of mechanical injury to starch particles in the milling process. It may be supposed that, as in the case of the artificially prepared soluble starch substrate, approximately 60% of this susceptible portion is saccharified during autolysis.

Alpha-Amylase

When extracts of *malted* or *germinated* wheat or barley are allowed to act upon soluble starch the amylolytic degradation is much more drastic and complete than is the case with extracts from ungerminated grain containing chiefly beta-amylase. This is manifested in several different ways: (1) Saccharification does not stop when about 60% of the starch has been converted to maltose. It may go to nearly 100% conversion. (2) The property of iodine coloration is completely lost, its *rate* of disappearance depending upon the concentration of malt amylase. (3) Complete loss of alcohol precipitable substance (dextrins, etc.) accompanies loss of iodine coloration. When gelatinized starch paste is used as substrate there is a rapid and progressive lowering of viscosity.

This more drastic activity shown by malt extracts, as compared with extracts of unmalted grain, is assumed to be due largely to the development, during germination, of an additional enzyme, designated as alpha-amylase, which acts in conjunction with the beta-amylase already present. It is of importance, therefore, to consider the individual behavior and the properties of alpha-amylase itself.

In contrast to the action of beta-amylase, which splits off maltose end-wise from the molecular starch chain until 60% has been converted, alpha-amylase is believed to effect an *internal* disintegration of the starch molecule, the first end products being dextrins rather than maltose (hence the designations of beta- and alpha-amylase, respectively, as the "saccharogenic" and "dextrinogenic" amylases). Acting by itself, with soluble starch as substrate, the immediate maltose-producing potentiality of alpha-amylase is of a decidedly lower order than that of beta-amylase, as can be observed by comparing data shown in Figures 1 and 2, on the one hand, with the results given in Figure 5 on the other.

For the experiment shown in Figure 5, the alpha-amylase was prepared according to the procedure used by Hanes (1937), and a portion of the dry preparation was dissolved in water. To 50-c.c. aliquots of 1% boiled starch solution were added, respectively, 2 and 20 c.c. portions of the alpha-amylase solution. The digests were then adjusted to pH 4.7 with acetate buffer, and made to 100 c.c. Aliquots

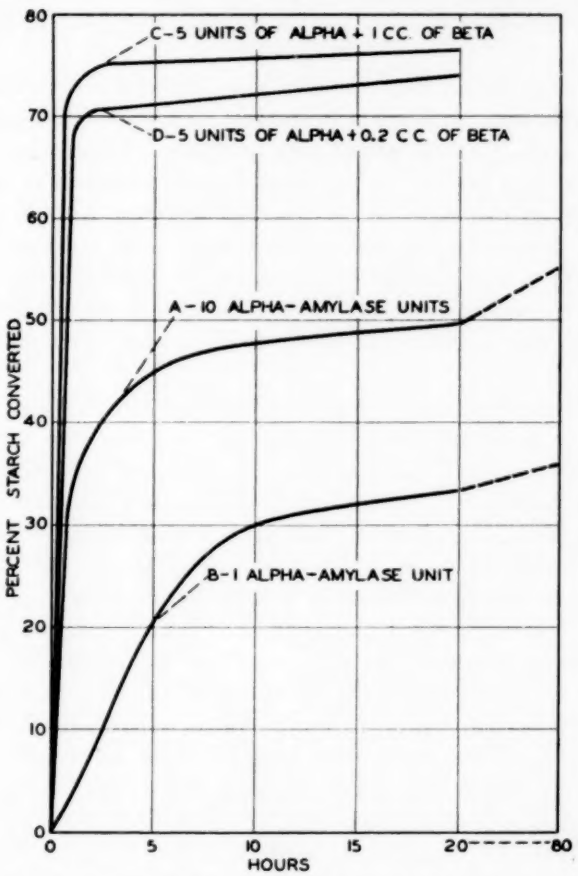

Fig. 5. Alpha-amylase acting alone, and with added beta-amylase.

of the digests were withdrawn at successive time intervals and analyzed for reducing substances (calculated as maltose) by the ferricyanide method. The curves in Figure 5, accordingly, show comparative rates of maltose production at two different concentrations of alpha-amylase, one concentration being 10 times that of the other. Figure 5 also shows results obtained with mixtures of individual preparations of alpha- and beta-amylase.

From Figure 5 it is seen that the saccharifying properties of alpha-amylase differ from those of beta-amylase (Figures 1 and 2) in two important respects. These are, respectively, *rate* and *degree* of starch conversion. In observing the relative saccharifying *rates* of the two amylases it is convenient to select, as a proper basis for comparison, conditions representing fairly high concentrations of the enzymes. In Figure 5, for alpha-amylase, this is curve A, while for beta-amylase any one of the three curves, 3.2 W, 0.8 W, or 0.2 W, in Figure 2 is suitable.

It has been shown that for any one of these three beta-amylase concentrations saccharification proceeded at a very rapid and uniform rate up to a point where about 60% of the starch was converted, at which point (reached in three hours) saccharogenesis abruptly and almost completely stopped. On the other hand, in the case of curve A, Figure 5, representing a comparably high concentration of alpha-amylase, rate of saccharification falls off more gradually, and is greatly diminished considerably before 50% conversion of the starch is reached —in fact, in this instance the 60% stage had not been reached in 80 hours.

With reference to *degree* of saccharification, the two amylases show very distinctive properties. The tendency for beta-amylase to effect approximately a 60% conversion, regardless of concentration, has been emphasized, and it has been shown that differences in concentration are registered chiefly in terms of time required to reach this degree of saccharification. For alpha-amylase, on the other hand, not only rate but also *degree* of saccharification seemingly depends upon the concentration of the enzyme. Whereas *all* concentrations of beta-amylase tend eventually to reach approximately the 60% conversion level regardless of time required, the degree of starch conversion by alpha-amylase appears to depend entirely upon the enzyme concentration. Thus in Figure 5, curve B never catches up with curve A. After the first few hours of comparatively rapid saccharogenesis, the rates of starch conversion become exceedingly low in both cases and curve B shows no tendency to approach curve A at any later stage. No satisfactory explanation for this peculiarity in the behavior of alpha-amylase has been established.

These distinctions between the two amylases, based upon studies of both rate and degree of starch saccharification, confirm in every way the findings and the conclusions discussed by Hanes (1937). The ability of malt amylases rapidly to destroy the iodine coloration property of starch, in contrast to the amylases of ungerminated grain, is a characteristic and distinctive property of alpha-amylase that needs little comment; indeed the rate at which this property is destroyed

has long been used as a basis for the quantitative estimation of alpha-amylase (Wohlgemuth method). Alpha-amylodextrin, which is the 40% fraction resistant to saccharification by beta-amylase alone and retains capacity for blue coloration with iodine, is obviously converted by alpha-amylase into smaller dextrins incapable of iodine coloration.

Curves C and D in Figure 5 show that the combined action of the two amylases, as prepared individually, results in much more complete and rapid saccharification than that produced by the action of either one alone. A detailed consideration of the behavior of the two amylases acting in combination is presented in a discussion of the action of malt extracts, which follows.

Combined Action of Alpha- and Beta-Amylases

Although it is highly desirable to know the individual properties of alpha- and beta-amylases, respectively, it seems quite unlikely that in dealing with cereals one would expect ever to encounter, under natural conditions, a situation in which the two amylases act entirely independently of each other. The nearest approach to such a situation, as already indicated, is found in wheat flour, a product of ungerminated grain in which the beta form is almost overwhelmingly predominant.

In so far as the cereals are concerned, only after germination is alpha-amylase found in relatively large quantities, and presumably it is always associated with an abundance of the beta form. Therefore it is obvious that when boiled, gelatinized, or other forms of *soluble* starch are digested with malt, the saccharification achieved is the net result of the combined action of the two amylases, not to mention certain other biocatalysts that may be involved.

It is of interest, therefore, knowing something of the properties of the two amylases, to observe their saccharifying powers when acting together in their natural state, and in varying amounts. For this purpose, of course, it is merely necessary to measure the progressive development of reducing sugars (calculated as maltose) when different amounts of a malt extract are allowed to act on soluble starch over a considerable time interval. In one set of experiments extracts of malted wheat and malted barley flour, respectively, were dialyzed over night and made to volume so that 10 c.c. was equivalent to 1 gm. of the original flour. One-hundred-c.c. portions of 1% Lintner starch solution were, individually, digested with 0.2, 0.8, and 3.2 c.c. aliquots of the enzyme extracts. The data from this series of experiments are presented graphically in Figure 6.

The data presented graphically in Figure 6 substantiate the values shown in Figure 5, in that a much more rapid and complete sacchari-

fication of the starch was effected by the combined action of the two amylases than by either one, when acting alone. With the two highest enzyme concentrations (3.2 MB and 3.2 MW) more than 75% of the starch was saccharified in one hour, whereas with beta-amylase alone (Figures 1 and 2) the limit of conversion was about 60% regardless of

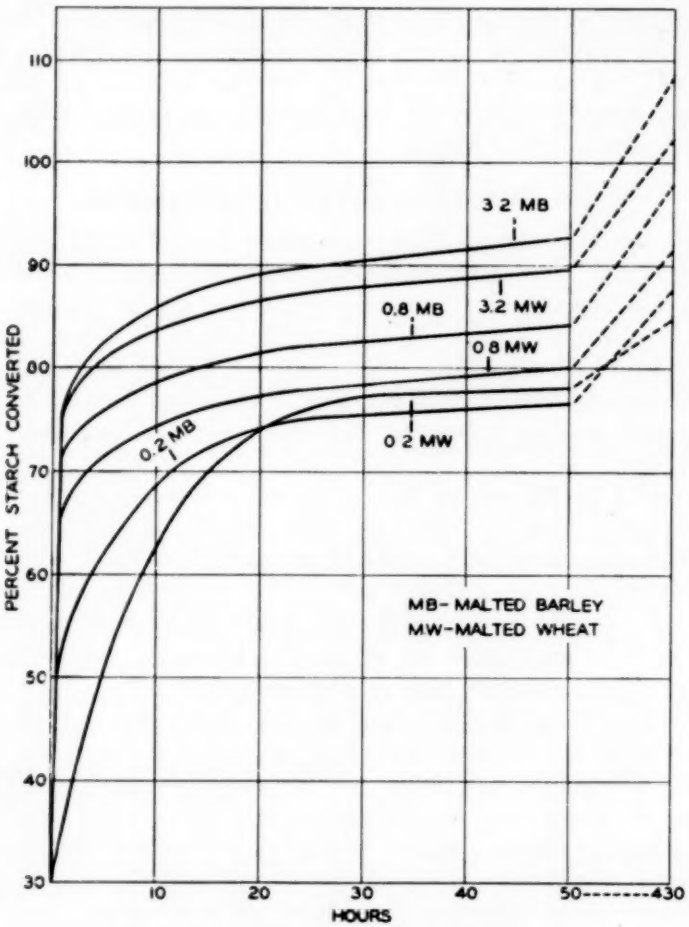

Fig. 6. Malted grain amylases acting on soluble starch.

enzyme concentration. It may also be observed that, unlike beta-amylase acting alone, there is no definite saccharification limit eventually reached by all concentrations but that *degree* of saccharification varies with the enzyme concentration. To this extent the data have quantitative significance, but obviously the variations in conversion levels are by no means *proportional* to the corresponding differences in

enzyme concentration. They merely show which preparations contained the greater amounts of alpha-amylase. In this respect the behavior resembles that of alpha-amylase acting alone (Figure 5).

It appears in Figure 5 that in the case of each of the two highest amylase concentrations a maltose conversion level of *more than 100%* was reached after very prolonged digestion. This can readily be explained by the fact that all of the sugar produced is not maltose, but that a considerable portion is glucose. Other workers, as mentioned by Hanes (1937) have reported that some glucose is produced by the action of malt amylase on soluble starch. This of course suggests the presence of *maltase* in the malt extracts. Since glucose has a much greater reducing power than maltose, it is certain that too high values will result when, with a mixture of the two, total reducing power is calculated on the assumption that maltose is the only reducing sugar present.

There is, therefore, the probability of considerable error in the prevailing custom of calculating as maltose all of the reducing sugars produced during starch degradation by cereal amylases. Under what conditions, respectively, does this practice involve the greatest, or the least errors? Experiences in this laboratory, using Barfoed's copper reagent—sensitive to glucose but not to maltose—lead to the following conclusions: (1) When beta-amylase alone acts upon soluble starch, even over prolonged time intervals, the amount of glucose produced is insignificant. (2) The same is true for alpha-amylase, alone, as prepared by a method involving the inactivation of beta-amylase according to Ohlsson's (1922) method. (3) When a mixture of individually prepared alpha- and beta-amylases acts on soluble starch, no glucose is produced in significant amount. (4) When extracts of *germinated* grain are used on starch, significant and sometimes very considerable quantities of glucose are produced, depending somewhat on the concentration or amount of enzyme extract used. Under such conditions, when the sugar produced is all calculated as maltose, values are certain to be too high, although they may be suitable for comparative purposes.

These experiences justify a presumption that the production of glucose during diastasis by malt enzymes is caused by maltase rather than by amylase activity. The entire subject will be considered more extensively in a later publication.

The discussion by Hanes (1937) clearly explains why the combined activity of the two amylases accomplishes a far more drastic and complete degradation of the soluble starch than does the individual action of either one, as typified by the experiments herein presented. It is only necessary to recall that one amylase (beta) attacks the mole-

cular starch chain in an end-wise manner, while the other (alpha) produces internal disintegrations that perhaps serve to expose additional end-groups to the action of the first-mentioned form. This viewpoint is supported by the fact that alpha-amylodextrin (erythrogranulose), which constitutes the 40% of original starch that is resistant to saccharification by beta-amylase, is readily degraded by alpha-amylase, and even more rapidly saccharified by the combined action of both.

Effect of Malt Amylases on Flour Behavior

From a consideration of the properties and activities of alpha and beta-amylase, both singly and in combination, it is possible to offer a plausible explanation of certain features of behavior observed when flour is supplemented with malt. It has already been shown that for sound, untreated flours autolytic saccharogenesis tends decidedly to give a rate curve of the beta-amylase type; that although flours normally contain large quantities of beta-amylase their *autolytic* maltose-producing capacities are frequently very restricted; and that beyond reasonable doubt the limiting factor is the amount of readily available ("soluble") starch present, together with the lack of alpha-amylase. Preliminary to an understanding of the supplementary effect of malt, it is only necessary to consider this substrate as strictly comparable to the soluble (boiled) starch used in the studies represented by Figures 1, 2, 5, and 6, which means that its very rapid initial rate of autolytic maltose production during the first hour or two is simply the expected manifestation of its partial saccharification by beta-amylase.

The immediate effect of adding malt, therefore, is to provide alpha-amylase, which supplements the action of the beta-amylase already present, thereby affording means for saccharifying the 40% (alpha-amylodextrin) fraction that was resistant to beta-amylase alone. Over extremely long time intervals saccharification may proceed considerably beyond these limits, as a result of an appreciable hydrolysis of *raw*, native starch, as will be discussed later.

It is a matter of common knowledge and experience that the increased activity produced by adding a small amount of malt falls considerably short of being doubled when the quantity of malt is doubled. In fact, as the amount of malt is progressively increased, the ratio of increased maltose production to malt added becomes progressively less. This might be expected in view of the limited quantity of substrate available and it is quite readily explained by the data in Figure 6, showing that increased saccharification due to added increments of alpha-amylase fall far short of being proportional to the progressively increasing quantities of enzyme used.

The results of a typical experiment showing the manner in which autolytic saccharification in a normal baker's flour is increased by additions of varying increments of malted wheat flour are graphically presented in Figure 7. In this experiment the values are recorded in terms of gassing power as measured by the yeast manometric method.

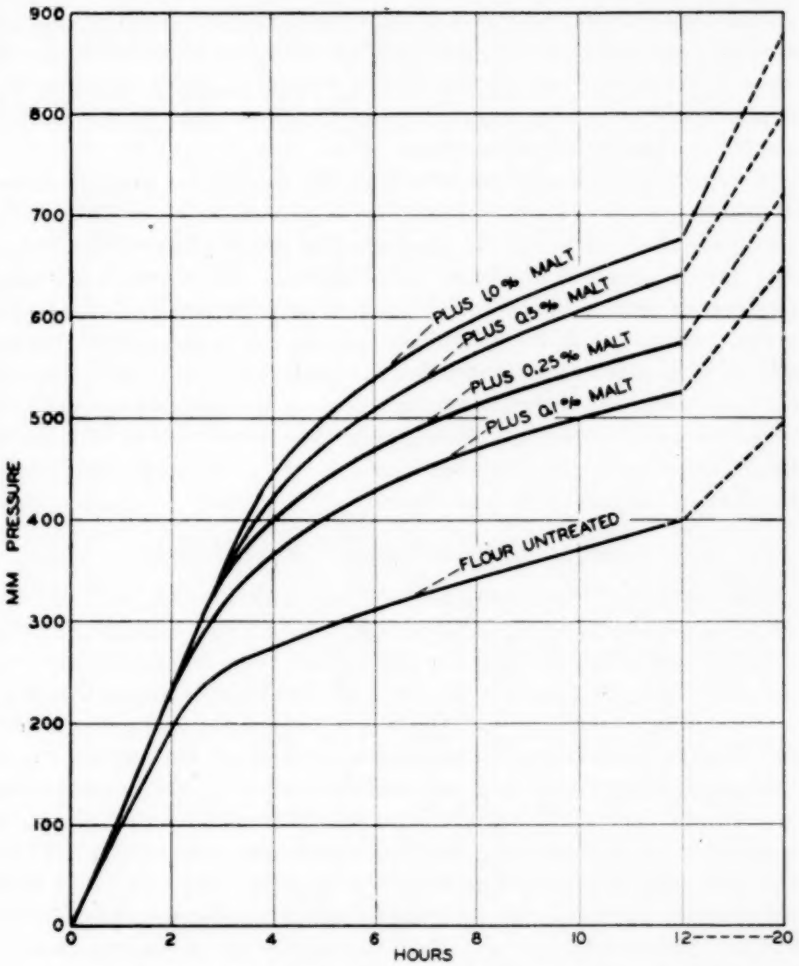

Fig. 7. Effect of malt upon flour autolytic saccharification—yeast manometric method.

The rate curves shown in Figure 7 display an *average* behavior, and they substantiate fully the viewpoints and interpretations herein proposed. All curves indicate a relatively high initial conversion rate up to a certain region of rapid decline, and from this region a much slower, though uniform, rate of saccharification is maintained. The region of

rapid decline of the initial conversion rate may be designated for convenience as the "transition region." In the complete absence of alpha-amylase, this region is supposedly reached at the 60% conversion level. However, as is clearly shown in Figure 7, the levels at which this region is reached become progressively higher as the increments of malt (alpha-amylase) become larger.

The rate curves show clearly that the increases produced by the varying increments of malt fall far short of being proportional to the amounts added. Thus, for example, 1% of the malted wheat flour was required to double (approximately) the effect produced by the addition of 0.1%, or one-tenth the amount.

From these findings and considerations it may be concluded that alpha-amylase is the most important factor that is contributed by malt flour when added in the amounts that are customarily employed in industrial practice. Munz and Bailey (1937) reached a similar conclusion from studies of dough plasticity as affected by malt supplements. In view of the abundant supply of beta-amylase always present in flour itself, together with the conversion limit (60%) beyond which beta-amylase cannot act, regardless of the amount present, it is not suprising that small additions of beta-amylase should be without significant effect, and that the alpha form is the important factor contributed by malted preparations.

Saccharification of "Raw" Wheat Starch

The alpha- and beta-amylases, respectively, are known and characterized on the basis of their hydrolytic effect on *artificially treated, soluble* (usually boiled) starch, and it has been emphasized in this discussion that the quantity of starch of this readily susceptible type, in wheat flour, is ordinarily quite small in proportion to the total starch substance. It therefore seems highly important to consider what, if anything, happens to the relatively great mass of raw, untreated flour starch during the autolytic process of dough fermentation. In this connection it may be recalled that while beta-amylase is the form occurring abundantly and predominantly in normal untreated flour, this enzyme, by itself, appears to be without significant action on raw starch. Nevertheless it is possible to show fairly conclusively that (1) during autolysis of most flours there is actually a measurable, though slow, biocatalytic degradation of the raw starch, (2) the rate of this conversion varies with different flours, (3) the differences are probably due largely to variations of an enzymic character rather than to differences in raw starch susceptibility, and (4) that (as might be expected) the raw starch-converting enzyme is many times more abundant in germinated than in ungerminated grain products.

The basis for the foregoing statements, as well as for the following discussion of the raw starch factor and its properties, is to be found in publications, respectively, by Standstedt, Blish, Mecham, and Bode (1937) and by Blish, Sandstedt, and Mecham (1937). Preliminary ideas as to the actuality, the rate, and the nature of raw starch degradation during flour autolysis may be reached from a study of autolytic saccharification rate curves for various flours under conditions that involve comparatively long time intervals.

Sandstedt, Blish, Mecham, and Bode (1937) reported upon comparative features of such rate curves obtained with six different flours. All showed the customary rapid initial saccharification rates up to their respective "transition regions," where the usual very conspicuous falling off in rate occurs, as shown in Figure 7. The curve up to this region, representing the rapid conversion of the readily susceptible starch by beta-amylase may for convenience be denoted as the "primary stage" of saccharification, and the *degrees* of saccharification reached in this primary stage for the various flours correlate closely with their respective quantities of suceptible starch, as shown by "maltose values," determined by the method of Blish and Sandstedt (1933). After passing through the "transition region," characterized by the rapid diminution in saccharification rate, the autolytic flour curves entered a so-called "secondary rate" of saccharification, in which a very much slower but *steady* rate was indefinitely maintained by each flour. Although each "secondary rate" flour curve progressed as a straight line, the lines had varying angles of slope, the steeper angles of slope indicating correspondingly higher rates of saccharification. A sample of soft wheat flour was found to have an almost negligible "secondary rate."

Since obviously the "primary stage" of rapid saccharification—denoted by the steep slope of the curve during the first two or three hours of autolysis—involves the conversion of the *readily susceptible* starch fraction, it was presumed that the much slower "secondary rate" must be essentially indicative of the rate at which the raw, native wheat starch is saccharified.

Did the differences in degree of slope in the secondary rate curves signify that the raw starches of different flours have different susceptibilities, or rather that the flours contained varying quantities of a biological factor that renders raw starch available to diastasis? It was found that when extracts of the flours were allowed to act, respectively, on portions of the *same* raw starch substrate, under comparable conditions, the differences in the slopes of the rate curves fell in exactly the same order as in the autolytic studies. Furthermore, when the flours were inactivated, enzymatically, and treated comparably with

portions of *the same* enzyme extract, the secondary rate curves were *parallel*. This showed that the raw starches of the flours had essentially the same degree of resistance, but that the differences in the autolytic studies must have been due to differences in amount of raw starch catalyst present.

Blish, Sandstedt and Mecham (1937) reported results of studies intended to disclose something of the nature and identity of the raw starch catalyst. It could be extracted from flour or malt along with alpha- and beta-amylase, but its separation from those amylases was not accomplished. Along with alpha-amylase it is far more abundant in germinated than in ungerminated grain, but, like alpha-amylase, it occurs also in ungerminated grain (flour) in significant though small and variable amounts.

When different concentrations of malted wheat and barley extracts (1, 5, and 10 c.c. aliquots of extracts prepared under comparable conditions), respectively, were allowed to act on 10-gram portions of raw wheat starch, the saccharification rates were as shown in Figure 8.

As contrasted with amylase action on *soluble* or boiled starch, the curves in Figure 8 do not show an extremely rapid initial rate of saccharification followed by a sharp change to a much slower rate. True, there seems to be a slight tendency toward such behavior but the rates are far more gradual and uniform from start to finish than with the *boiled* starch substrate. Both rate and degree of saccharification vary with enzyme concentration, and the lower concentrations never catch up with the higher ones.

It appears to be generally believed, as stated in the introductory portion of this report, that *raw* starch diastasis is made possible by the action of a cytase (hemicellulase?) that gradually destroys the "cellulose" wall enclosing the starch cells, thus exposing the soluble inner portion to the action of the amylases. According to this theory, it would perhaps seem reasonable to expect a very slow if not an almost negligible *initial* saccharification rate up to the point at which the cellulose wall is destroyed by the cytase—this to be followed by a rapid rate of conversion such as is characteristic of the first stages of *soluble* starch hydrolysis. The rate curves in Figure 8, however, do not seem to support such a supposition; in fact they indicate a behavior that is incompatible with the generally accepted hypothesis. They suggest a fairly homogeneous character of the raw starch substrate.

These findings, supplemented by a few microscopic observations, tend to support the views of Brown and Heron (1879), who reported that the initial stages of *raw* starch diastasis are characterized by the

appearance of small localized perforations or corrosions of the outer covering of the starch cells. Penetration through these perforations by the amylases progresses slowly and gradually, until finally nothing is left but the remains of the enveloping structure. The rate curves in Figure 8 are somewhat in harmony with this theory, but offer no suitable explanation as to why the lower enzyme concentrations show

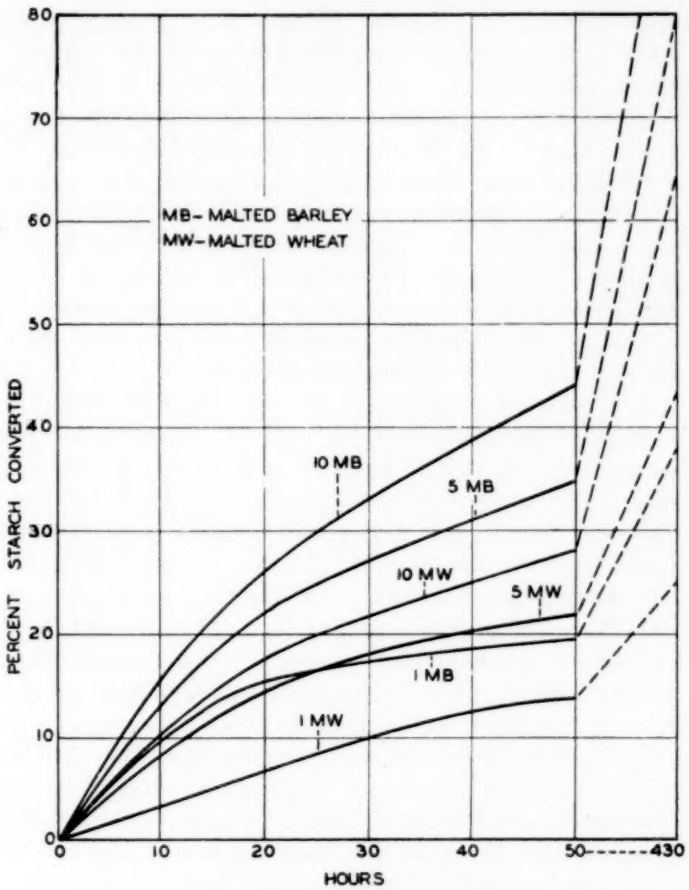

Fig. 8. Malted grain enzymes acting on raw wheat starch.

no tendency eventually to "catch up" with the higher ones after long time intervals. Only with the highest enzyme concentration used (curve 10 MB) did the saccharification proceed near to completion. Here, however, as was the case with the results in Figure 6, the final values are known to be too high owing to the fact that considerable glucose (calculated as maltose) was formed during the digestion.

The exact nature and identity of the raw starch catalyst will perhaps remain doubtful until it can be separated from other enzymes and studied as an individual. Apparently this has not been accomplished. Since both alpha-amylase and the raw starch factor are both produced abundantly during germination, there is every reason to suppose that the two factors have some properties in common, and our preparations of alpha-amylase always seem to contain significant amounts of the raw starch catalyst. This makes it difficult, if not impossible, to show whether alpha-amylase, *alone*, is or is not active on raw starch.

Lacking direct proof, it is nevertheless possible, by means of circumstantial evidence, to establish the strong probability that alpha-amylase and the raw starch catalyst are separate enzyme factors. For example, Blish, Sandstedt, and Mecham (1937) were able to prepare from malted wheat flour a non-enzymic substance that under certain conditions served to stimulate raw starch saccharification but the "activator" did not effect a corresponding stimulation of alpha-amylase activity. It was found also that dialysis of a malt extract will frequently show a stimulating effect on the rate of raw starch saccharification, but not on alpha-amylase activity.

Although there is a pronounced tendency for malts high in alpha-amylase to be correspondingly high in the raw-starch catalyst, and *vice versa*, instances of lack of parallelism between the two catalysts should constitute additional evidence that they are separate and distinct factors. Using the Wohlgemuth iodine method for alpha-amylase activity, on the one hand, and the saccharification-rate method for the raw-starch factor, on the other, in testing various series of malt samples, definite instances of lack of parallelism have been noted. However, we have not gained sufficient confidence in the quantitative precision of the Wohlgemuth method to warrant a presentation of data bearing on this issue.

Significance of the raw starch catalyst in baking.—There seems to be no reason to regard the raw-starch catalyst as a factor of serious consequence in ordinary baking procedures. In cases involving additions of malt, either to the flour or to the dough, it may be assumed that the sugars produced by diastasis of the *readily susceptible* starch will be supplemented to a measurable degree by a slow saccharification of the *raw* flour starch. Although the amount of additional sugar thus produced is perhaps relatively unimportant in normal baking practice, the quantity may be considerable under conditions involving extended fermentation periods.

Variations in Malt Diastatic Power

In view of the established practice of measuring and designating the saccharifying powers of malts in terms of their respective *Lintner values* it is of interest, in so far as possible, to account for variations in Lintner value on the basis of the individual and combined properties of the alpha- and beta-amylases. Obviously the generally accepted belief is that the Lintner method affords primarily an exclusive measure of beta-amylase activity. While this is undoubtedly true for ungerminated cereals or cereal products, it would seem from a consideration of Figures 5 and 6 that in the case of *malt*, alpha-amylase might easily be an important contributing factor.

With both amylases present, it may be expected that the percentage of starch converted would in time go considerably beyond the 60% level, which is the limit for beta-amylase alone. Over an extended digestion period with a soluble starch substrate those malts having the higher alpha-amylase contents should reach these higher conversion levels more quickly than those of lower content. However, the Lintner method, involving a relatively small quantity of malt, establishes conditions whereby the 60% level of starch conversion is not reached in the prescribed digestion period of one hour. This might lend support to the belief that differences in Lintner values primarily reflect differences in beta- rather than alpha-amylase activity, but curves C and D in Figure 5 show that in the presence of equal amounts of alpha-amylase the quantity of beta-amylase was varied from 0.2 to 1.0 unit without more than a trifling effect upon the degree of saccharification reached in one hour. It therefore is reasonable to suspect that differences in Lintner values may in many instances serve to indicate variations in alpha- as well as in beta-amylase. In fact our own observations with malts of known Lintner values appear strongly to substantiate this viewpoint.

In an attempt to investigate the meaning of Lintner values in the light of this and other considerations, six malts of varying known Lintner values [3] were studied and evaluated as to their various starch-degrading activities. The malts were ground on an ordinary coffee-mill type of feed grinder. Each sample of ground malt was extracted over night with water, using one part of malt to 10 parts of water, with toluol as a preservative. The properties studied were (1) activity on boiled potato starch,[4] (2) activity on raw wheat starch,[5] (3) autolytic saccharification,[6] and (4) alpha-amylase activity.[7] The data obtained are shown in Table I.

[3] The malt samples, with their Lintner values were kindly furnished by Dr. D. A. Coleman.
[4] 50 c.c. of 1% starch solution, plus 1 c.c. of malt extract, buffered to pH 4.7 and made to 100 c.c.
[5] 10 gm. starch plus 10 c.c. of malt extract, suspended in 100 c.c. of water at pH 4.7.
[6] Total reducing sugar (as maltose) in a 1-to-10 water digest of malt, over night.
[7] Wohlgemuth iodine method.

There is a pronounced tendency for the values for each property shown in Table I to fall in precisely the same order as the Lintner values. If the Wohlgemuth method is acceptable as a reliable quantitative measure of alpha-amylase activity, there is indeed a very high correlation between the alpha-amylase and the Lintner values. This offers a convincing argument against the belief that Lintner value is exclusively a manifestation of *beta-amylase* activity. With one exception the autolytic values follow the same order as the Lintner numbers, while the same is true of *raw starch* saccharification.

From the standpoint of the industrial utility of malt, our main concern presumably is with rate and degree of saccharification of the boiled starch substrate, regardless of the parts played by each individual catalyst. Under the conditions of the experiments reported in

TABLE I

STARCH-DEGRADING PROPERTIES OF VARIOUS MALTS

Sample	Lintner value	Boiled-starch conversion			Raw starch conversion[1]	Autolytic values[2]	Alpha amylase[3]
		15 min.	1 hr.	24 hrs.			
	Degree	%	%	%	*Mg. maltose*		
A. Illinois 6 row	93.6	44.4	74.0	86.6	825	211	100
B. Michigan 6 row	86.5	40.0	72.4	85.8	750	191	88
C. California 6 row	50.5	24.4	67.2	83.6	665	133	48
D. Oregon 2 row	29.5	15.9	56.8	80.8	545	145	44
E. California 6 row	25.4	14.5	50.0	81.0	640	135	38
F. Oregon 2 row	23.3	12.8	48.4	79.4	425	125	36

[1] Difference between values at 6½ hours and at 24 hours.
[2] As total maltose present after 16 hours autolysis at room temperature.
[3] Calculated on basis of assigning a value of 100 to sample A.

Table I, the highest correlation (both in order and in magnitude) with Lintner values is found in the data showing degree of starch conversion in *15 minutes*, It is of particular significance, however, that as the digestion time is prolonged the range of values indicating *degree* of saccharification becomes progressively narrower. Thus, if we consider the two extremes of the six samples for the 15-minute period, sample A shows a value about 3½ times greater than F. At one hour A is about 1½ times greater than F, while at 24 hours the values for A and F are 86.6 and 79.4 respectively.

Table II gives similar data obtained with five malt samples furnished by the A. A. C. C. Committee on Malt Analysis, the method of procedure being the same as used in experiments reported in Table I.

As with the data presented in Table I, Table II shows that the greatest differentiation among the saccharifying powers of the malts

is afforded by the 15-minute values, which perhaps would correspond closely with the actual Lintner values. The malts represented in Table II involve a higher range of activities than those in Table I. With this fact in mind it is of interest to compare the one-hour values in the two series of malts. In Table I, the one-hour values are, to be sure, much closer together than are the 15-minute values. In Table II, however, the one-hour values are—excluding sample No. 1—nearly *identical*. This would suggest that in the higher range of Lintner values variations shown in *initial* saccharifying activity disappear more rapidly on continued digestion than is the case with malts in the lower Lintner-value ranges.

The above considerations provide justification for the doubts entertained by many technologists as to the practical value and utility of Lintner determinations for brewing purposes. The

TABLE II

DEGREE OF SACCHARIFICATION AT PROGRESSIVE TIME INTERVALS

Malt sample	Boiled starch conversion			Estimated Lintner values[1]
	15 min.	1 hr.	24 hrs.	
	%	%	%	
1	25.2	69.8	83.0	53
2	38.6	73.6	87.0	82
3	48.0	73.0	84.6	103
4	57.2	74.0	84.8	126
5	61.6	74.4	85.8	136

[1] Estimations based upon 15 minute saccharification values, in accordance with previous experiences with malts of known Lintner values.

specifications of the Lintner method apparently take chiefly into account the *initial stages* of saccharification, in which malts show differences that would tend to disappear under conditions that prevail in the actual mashing process, where starch conversion is carried to a much higher degree of conversion. Thus, under the experimental conditions represented in Tables I and II, it is apparent that the one-hour values will more reliably indicate the relative saccharifying powers of the malts under the conditions of *actual brewing operations* than will the 15-minute values, despite the fact that the latter are in better agreement with the Lintner values.

Yeast Manometric Method for Malt Amylase Activity

The yeast manometric procedure established by Sandstedt and Blish (1934) as suitable for measuring differences in rate of diastasis among wheat flours was subjected to a brief preliminary study as to its applicability in the diastatic differentiation of malts. In exploring

the possibilities of this type of method, the five malt samples represented in Table II were used in the manometric apparatus ("pressure meters") devised by Sandstedt and Blish (1934).

Fresh baker's yeast having been shown (Sandstedt and Blish, 1934) to be variable and generally deficient in its ability to ferment maltose in the absence of certain unidentified biological "activators" it was found necessary to employ the use of activator substance in order that the yeast might ferment the maltose produced by the action of the malt on the boiled starch. Although malt itself contains the activator, the amount of it that must be used in this type of method is so small that the quantity of activator supplied from that source alone is insufficient to stimulate adequate yeast fermentation. The activator used in these preliminary experiments was the *dried* yeast preparation described by Blish and Sandstedt (1937).

The results with the five malt samples represented in Table III were obtained by the following procedure: To 50 c.c. of 1% boiled

TABLE III

YEAST MANOMETRIC METHOD FOR MALT DIASTATIC POWERS

Malt	Pressures—30° *mm. of mercury*				Pressures—40° *mm. of mercury*				Estimated Lintner values	Alpha-amylase index
	2 hrs.	4 hrs.	7 hrs.	22 hrs.	2 hrs.	4 hrs.	7 hrs.	22 hrs.		
1	6	20	81	236	6	45	122	320	53	34
2	8	34	116	260	13	57	140	338	82	58
3	11	62	145	267	19	75	157	340	103	67
4	13	76	156	273	26	90	178	342	126	85
5	17	83	166	282	31	101	181	347	136	100

starch solution in the aluminum pressure cup were added 10 c.c. of a suspension containing 1 gm. of fresh yeast and 1 gm. of the dried yeast "activator" preparation, and 2 c.c. of the malt extract. The malt extract was prepared by treating 1 gm. of ground malt with 200 c.c. of water; therefore the 2 c.c. portion of this extract used represented only 0.01 gm. of dry malt. The restriction of the malt to a very small quantity is desirable in order to prevent the production of excessive quantities of maltose before active yeast fermentation begins. This provision tends to promote the most favorable conditions for differentiation among the malts within a reasonably short time interval. The suspension containing 1 gm. of fresh yeast and 1 gm. of activator per 10 c.c. of water was allowed to stand two or three hours before use, in order to ferment out substances that otherwise might give higher "blank" values than are desirable.

After thoroughly mixing the yeast, activator, malt, and starch solution, the lid holding the mercury manometer was screwed tightly on the cup, which was immersed in a water bath. After allowing five minutes for the apparatus to reach the temperature of the water bath the pressure was equalized with the atmospheric pressure by opening the valve for an instant, following which the starting time was noted, and manometers were read at progressive time intervals.[8] Table III gives values obtained by the above procedure, in two series of tests, one operated at 30° and the other at 40°.

It may be observed in Table III that during the first few hours the values fall in the order of the Lintner and alpha-amylase values, respectively. As in the case of the saccharification-rate values obtained by the ferricyanide method for reducing sugars, the range of values becomes narrower as the digestion time is increased. The range of malt values for any given time interval is found to be wider at 30 than at 40 degrees. This has practical significance when it is considered that mashing operations are generally conducted at relatively high temperatures. The experiments suggest the applicability of this type of procedure in evaluating malts. It should be possible to select a set of conditions, including time, temperature, proportions of ingredients, etc., that will best serve any purpose for which the test might be conducted. The use of the maltose fermentation "activator" is a necessary feature of the procedure, for without it there is no assurance of active and satisfactory fermentation of the maltose by ordinary baker's yeast. It is quite likely, however, that the quantity of dried-yeast activator used in the foregoing experiments was considerably in excess of the amount actually needed.

The possibilities of the yeast manometric method justify further and more detailed investigation. Although under conditions herein reported it does not yield results as quickly as the chemical method, it nevertheless is convenient in that it requires very little of the technician's time and attention. It offers an advantage in that observations (manometer readings) can be made rapidly for any desired time interval.

Summary and Conclusions

Beta-amylase, the so-called "saccharogenic" amylase, occurs abundantly and predominantly in ungerminated cereals, and in wheat flour. Most flours also contain small, variable quantities of alpha-amylase and of a biocatalytic factor that promotes a relatively slow diastasis of *raw* starch.

[8] Shaking the pressure cups had to be avoided, since it promotes the release of dissolved CO_2 to such an extent that a constant reading is not possible.

Beta-amylase is without significant action on raw, native wheat-starch granules. Its distinctive property is its saccharifying action on starch that has been rendered available ("soluble") by physical, chemical, or biological means. Its action ceases when approximately 60% of such a starch substrate has been saccharified. The remaining and resistant 40% is a dextrin called "alpha-amylodextrin," which retains its property of being colored blue with iodine. The greater the concentration of beta-amylase, the more rapidly will the 60% conversion level be reached.

The important factor limiting the degree of *autolytic* saccharification in flour is the quantity of this readily available starch present in the flour. Flours contain small, variable amounts of this susceptible starch, the major portion of the total starch being intact starch grains unavailable to beta-amylase. The quantity of *available* starch can be increased by fine grinding, or by the addition of the raw starch biocatalyst, which occurs most abundantly in malt. It appears safe to assume that autolytic flour "maltose values" represent approximately a 60% conversion of the *available* starch fraction.

Alpha-amylase, the so-called "dextrinogenic amylase," permits the saccharification of "*soluble*" starch to proceed beyond the 60% conversion level, and the greater the concentration of alpha-amylase (other factors being equal) the higher the conversion level. *Degree* of increased saccharification is not directly proportional to increase in alpha-amylase concentration, the behavior following the law of diminishing returns. This explains the fact that doubling the quantity of added malt does not double the increase in saccharification.

The addition of malt to wheat flour accomplishes two things. First, it furnishes alpha-amylase, thereby permitting saccharification of the susceptible starch fraction to proceed beyond the 60% level afforded by beta-amylase alone. Second, it provides a catalyst that stimulates an appreciable saccharification of the *raw* starch.

Although the amount of alpha-amylase tends to parallel the quantity of the raw starch catalyst in malt, there is good evidence indicating that the two catalysts are not identical.

There is a tendency toward parallelism between Lintner value and alpha-amylase content in malts. Methods of the Lintner type, which take into account only the *initial stages* of saccharification, show much greater differentiation among malts than appears when saccharification is permitted to proceed further, as is apparently the customary practice in actual mashing operations in brewing. Analytical methods of the latter type should therefore afford a more rational basis for evaluation than does the Lintner method.

The yeast manometric type of method appears to have possibilities as a suitable means for estimating the diastatic potentialities of malt. It is necessary to use a maltose fermentation activator in a procedure of this type.

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REMOVAL OF THE BRAN FROM CEREALS

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(Received for publication May 24, 1938)

The removal of the bran from grain has always been something of a problem to the miller, baker, and others. There are conditions, however, when the complete removal of bran and other coverings of the wheat kernel is desirable, such as when it is sought (1) to determine the quantity of bran to endosperm in different varieties of grain and under different soil conditions, (2) to note the degree of horniness or starchiness in the grain when the cover is off and the endosperm color is exposed as is possible only when the bran is removed, (3) to be able to produce a special flour with the bran all out and all the germ and endosperm present, and (4) to produce another wheat breakfast food or hominy where the germ and oil have not been removed or crushed and therefore its oil less subjected to oxidation and the development of rancidity.

Review of Literature

Winton (1937) calls attention to the need of separation of bran for study before grinding as follows: "Grinding the whole kernel is as unscientific as to make no attempt before analysis to separate several minerals in the same matrix." Cobb (1905) made an effort to separate by hand five arbitrary zones of a wheat grain by painstakingly scraping the grain of plump purple-straw wheat kernels in such a way as to produce layers from five concentric zones (Figure 1). The flour from each zone was then analyzed for gluten and it was found that the percentage decreased progressively from the outer to the central part of the endosperm as follows: 16.5, 13.9, 9.5, 8.6, and 7.4% protein for each of the different zones. Fleurent (1896) also tried to separate the bran mechanically from Russian, Algerian, and Canadian wheat and reports 13.05, 13.51, and 13.01% respectively for the wheat from these countries. The reported percentages, however, look as if they might be from check samples. The writer has obtained considerable variation in the amount of bran present in different varieties and even in the same wheat grown on different soils.

The fibrous protective coverings of the wheat berry are called pericarp, testa, and perisperm. The reddish-brown pigment which characterizes many wheats is contained in the testa. The aleurone

layer is usually removed with the bran in milling, as it adheres to the perisperm. The term bran is so indefinite, as Winton comments, "as to render much published data of little value. Some milling processes run together germ, true bran (I use the term arbitrarily for fruit coat, seed coat, and hyaline layer) aleurone cells, and more or less starchy endosperm." It is evident from the above statement that a more definite separation is desirable in grading grains.

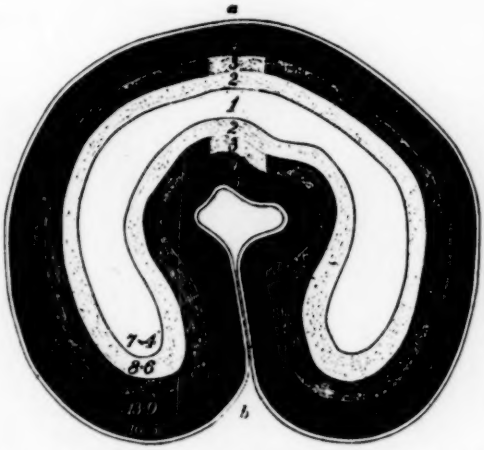

Fig. 1. Diagram of cross section of a wheat grain showing the gluten content of five arbitrary "zones." (Cobb, "Universal Nomenclature of Wheat.")

Method of Separation

The method of separation as carried out in this study was as follows: Fifty grams of grain which had been dried to constant weight at 100° C. were treated with 8 c.c. of sulphuric acid (sp. gr. 1.84). This was stirred for two minutes to coat each grain with the acid, and this stirring was continued each minute for 10 minutes or until all the seed coats had been blackened—which requires about 10 minutes for wheat and 20 minutes for corn at room temperature of 25° C. The blackened grains were next treated with three cubic centimeters of nitric acid (sp. gr. 1.42) added drop by drop and stirred constantly, as the chemical action of nitration is very rapid at this stage of treatment. As the temperature rises the black grains turn yellow, some brown fumes come off, and a yellow layer resembling whipped cream gathers on the top of the grain as the nitration continues. This process requires from two to three minutes depending on the kind of grain treated. The wheat and bran layers were next treated with 300 c.c. of distilled water with constant stirring and the bran residue was decanted off through a Buchner funnel (15 cm.) using a dried and

weighted paper filter. The grain was washed with water until 1500 c.c. of wash water had been obtained or until the water was no longer acid. The water was then decanted off and the grain was dried in an oven to constant weight at 100° C. It was again weighed and the loss of weight from the original 50 grams was calculated as bran. The amount of reducing sugar was determined in the 1500 c.c. of wash water by use of Fehling's solution and the weight of cuprous oxide produced was calculated as invert sugar. The filter paper containing the undissolved bran residue also was dried and weighed and the percentage of residue noted.

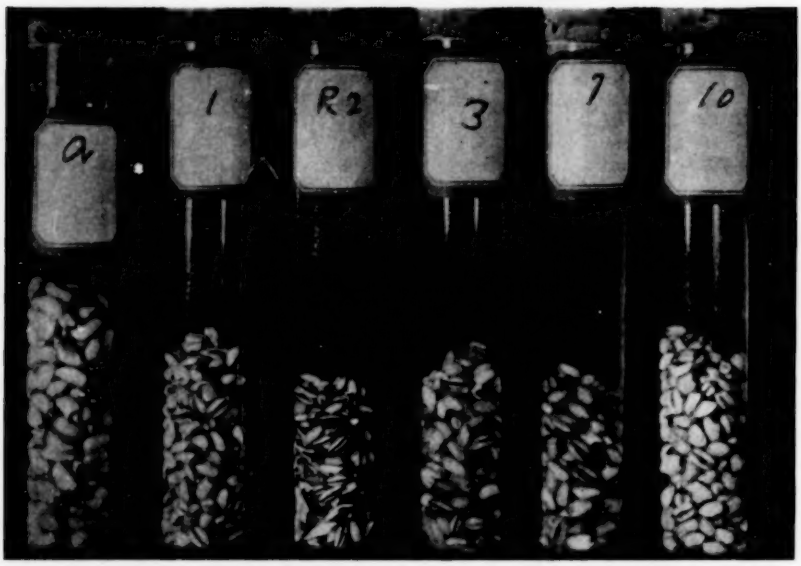

Fig. 2. *A* = white popcorn—bran removed; 1 = Michikof wheat—bran removed; R_2 = rye—bran removed, small endosperms appear; 3 = durum wheat—bran removed, white and yellow grains shown; 7 = 21–2–11 wheat—bran removed, white and yellow grains shown; 10 = white wheat—bran removed.

The grains were finally freed from all bran layers and appeared white—except where "hard" wheat or yellow corn had been used. In the case of hard horny wheat the color will be yellow and the corn also will be somewhat yellowish as may be noted in Figure 2. In order to study the action of the sulphuric acid alone on the dry grains and on the moist grains, samples were treated with water after the grain had been blackened but no nitric acid added. It was found that over one-third of the weight of the bran had been converted to a water-soluble form by this treatment and could be washed off the grain, leaving it with its near natural color. The soluble part, however, was found to be about two per cent less where the grain had been previously

moistened than where the sulphuric acid had been added to the dried grain. In the moistened-grain treatment it was aimed to use the equivalent of 72% sulphuric acid commonly used for dissolving the cellulose from plant compounds. The data obtained on several kinds of grain are given in Table I.

TABLE I

THE BRAN LAYERS OF SOME GRAINS WITH ASSOCIATED MATERIALS

Grain	Bran, H_2SO_4 and HNO_3 treatment	Cellulose, H_2SO_4 treatment	Invert sugar in bran solution	Bran–acid, insoluble residue	Protein, whole grain
	%	%	%	%	%
1. Michikof wheat	15.06	6.10	1.85	1.94	10.05
2. Trumbull wheat	13.72	4.84	1.60	1.88	8.84
3. Durum wheat	14.09	5.37	1.60	2.01	16.78 yellow 12.54 white
4. Poole wheat	13.39	4.70	1.32	2.16	8.25
5. Kanred wheat	11.75	2.79	1.60	1.69	9.57
6. Fultz wheat	12.32	3.86	1.87	2.01	10.15
7. 21–2–11 wheat	13.36	4.81	1.60	2.17	12.54
8. Rudy wheat	13.53	3.69	1.87	2.01	10.72
9. Hard winter wheat	15.50	5.86	1.50	2.19	11.91
10. Hard white wheat	14.92	6.28	1.60	1.92	11.23
11. Rye I	17.46	—	1.20	3.68	10.26
12. Rye II	25.60	16.38	1.58	4.01	10.62
13. Soybean	17.06	4.44	2.42	2.72	38.00
14. Corn—yellow	12.81	4.93	0.59	1.30	10.20
15. Corn—yellow pop	13.01	4.93	0.79	1.92	10.37
16. Corn—white pop	13.97	3.43	0.52	1.27	10.62

Summary

A method is given for the complete removal of bran layers from grains by chemical means. This has been done previously only by mechanical means—so far as the writer is aware.

The removal of the bran layers makes it possible to examine the color and texture of the endosperm and study the bran composition, and the comparative quantities of bran and endosperm. The latter is of special importance with rye because of large variation.

The chemical removal of the bran depends upon the selective action of a small amount of strong sulphuric (8 c.c.) for a short time (10 minutes). This prepares the way for the nitration of the cellulose with a small quantity of nitric acid (3 c.c.). The action is rapid (2–3 min.) but is soon stopped by the addition of water. This treatment also makes possible the separation of the heavy grains from the light, insoluble bran residue by means of gravity.

The quantity of bran varied from 11.75% in Kanred wheat to 15.06 in the Michikof variety. The bran content was more variable in rye,

ranging from 17.46 to 25.60 in the samples available. The insoluble residue in the acid-water treatment of the bran varied from 1.69 in wheat to 4.01% in rye. A part of this residue was somewhat wax-like and would float on the top of the wash water, whereas the remainder would sink to the bottom of the container. Only a small quantity of carbohydrate was involved in this process, as the reducing sugars varied from only 0.52% in popcorn to 1.87% in Rudy wheat.

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THE USE OF A PURIFIER IN EXPERIMENTAL MILLING

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(Received for publication February 4, 1938)

Willard and Swanson (1911) described a system of experimental milling with a pair of corrugated rolls, a pair of smooth rolls and a separate rotating sifter, all very similar to the equipment still widely in use on the American continent. Although purifiers are generally considered important machines in modern commercial milling they are rarely part of the experimental milling equipment. It is interesting to note that Willard and Swanson (1911) used a purifier, of which they gave details of construction. Their purifier worked with air separation alone, *i.e.*, without any sieves. The results did not seem to warrant the extra time and increased complication so that later the use of the purifier was dropped.

The only other reference to a purifier for laboratory work was found in a paper by Scharnagel (1935) dealing with the milling and baking quality of various wheat varieties.

Barbade (1934) made an investigation of milling on a small scale and gave details of his flow sheet and method of milling. The diameter of the rolls was 200 mm., which is larger than the size commonly used for laboratory work with relatively small samples (150 mm.). Kranz (1935) aimed at a 60% extraction (0.46% ash on a 13.5% moisture basis).

Geddes and West (1930) made a thorough study of the reliability of the experimental milling test. They state that "the first milling deviates more from the mean than any of the other millings of the day." Cold rolls give a higher yield and they advocate warming up the rolls before testing. Furthermore, they note that the temperature of the mill room has a greater effect on flour yield than relatively small variations in humidity.

The following are a few of the different systems of milling used:

	Number of Breaks	Number of Reductions
Willard and Swanson (1911)	5	21
Geddes and West (1930)	5	8
Barbade (1934)	4	4
Brabender automatic mill	1	1
Kranz (1934, 1935)	3	3
Buhler automatic mill (1935)	3	3
Geddes and Aitken (1937)	5	8

The experimental milling test is useful (Herman, 1927) but has its limitations. Bailey and Markley (1933) state that as a result of their tests the agreement in milling yield between a commercial mill and experimental mills in 2 different laboratories was "not all that might be desired."

Swanson and Kroeker (1932) draw attention to the fact that, compared with other laboratory methods, very little work has been done toward improving methods of making milling tests on wheat. Markley and Treloar (1937) attribute this to the large variation in the results of replicated bakings, so that there is no call for more uniform results in experimental milling at present. Barbade, Pisani-Borg and Duval (1935) believe that the limitations of the test are due to bad milling equipment.

As to the results obtained with experimentally milled flours compared with commercially milled flours, Pascoe *et al.* (1930) found that the absorption of the experimental flours was invariably lower. This is no doubt related to the coarser granulation of many flours produced in the laboratory. Naszalyi (1935) published results showing that flours obtained in the laboratory usually have a smaller extensibility, as measured by the Chopin extensimeter, than flours from a commercial mill.

In almost all the literature on the characteristics of experimentally milled flours the statement is found that these flours have a much lower diastatic activity than commercial flours from the same wheats. A list of some published results will be given in the experimental part of this article. Blish and Sandstedt (1927) found that the lack of stability in baking experimental flours as compared with commercial flours was due to the low diastatic activity of the former. Kent-Jones (1927) drew attention to the danger of milling too dry, which, according to Markley and Bailey (1934), results in high diastatic activity. The latter also state that much of the variation in the diastatic activity of experimental flours is occasioned before the flour is milled, *i.e.*, during tempering. During the grinding and bolting they also found that the condition of the surface of the flour particles is affected by the humidity of the air; the lower the relative humidity the higher the diastatic activity.

Sandstedt *et al.* (1937), in studies on the mode of attack on starch by the amylases, stated that commercial flours owe their higher diastatic activities to the fact that they contain relatively larger amounts of the more susceptible starch fractions (*i.e.*, contain more ruptured starch granules)—not to any difference in enzyme nature or content.

Geddes and Frisell (1935) described a small mill which can satis-

factorily handle 100 g. of wheat, as shown by the results of Geddes and Aitken (1935).

According to Mueller (1934), the Brabender automatic laboratory mill was not designed to produce middlings, but flour straightway, having a constant ash content, whether the yield be 10 or 60%. In a footnote Geddes and Aitken (1937) gave a statement by Brabender that "the fundamental purpose of his mill is to supply flour in the shortest time for farinograph testing and not primarily to yield flours comparable to those produced by commercial mills." The Canadian authors give mean yield values for the Brabender and the Allis-Chalmers mill of 47.3% and 67.9%, respectively, with mean ash contents of 0.63% and 0.43%. A further step in the development of the experimental milling test is the introduction of another automatic mill, the Buhler, described herein.

Description of Buhler Mill

The Buhler mill is pictured in Figure 1. The main parts of the mill are one pair of rolls for the three breaks each with a different corrugation and one pair for the three reductions. The roll length of each of the six streams has been chosen according to the relative amount of stock normally delivered by the feed gate. The feed to the first break from the wheat hopper can be regulated to admit anywhere from 10 to 50 pounds of wheat per hour, although for testing purposes 20 to 25 pounds per hour represent a normal speed.

After passing the rolls, the stock is bolted over wire and silk, the sifter doing a to-and-fro motion. The flour leaves the mill immediately; the middlings are led through a spout and elevated to the first reduction. The tails of the wire drop into one of the five elevators and are conveyed by a special belt to the second break. The tails from the third break are considered as bran, which leaves the mill at the back of the machine.

On the reduction side the middlings are treated in much the same way. The tails of the silk below the third reduction leave the mill as offal.

Mills are built either to deliver 2 flours—a mixture of break flours and a mixture of reduction flours—or to deliver 3 separate break flours and 3 separate reduction flours. The silks are chosen according to the type of flour needed.

The setting of the rolls is done by micrometer screws, the opening of the rolls being indicated on the 4 scales seen in Figure 1. Each mark on the scale is equivalent to 0.005 mm.

The ash content of some products will be found in the experimental section of this paper. The following figures show the diastatic activity

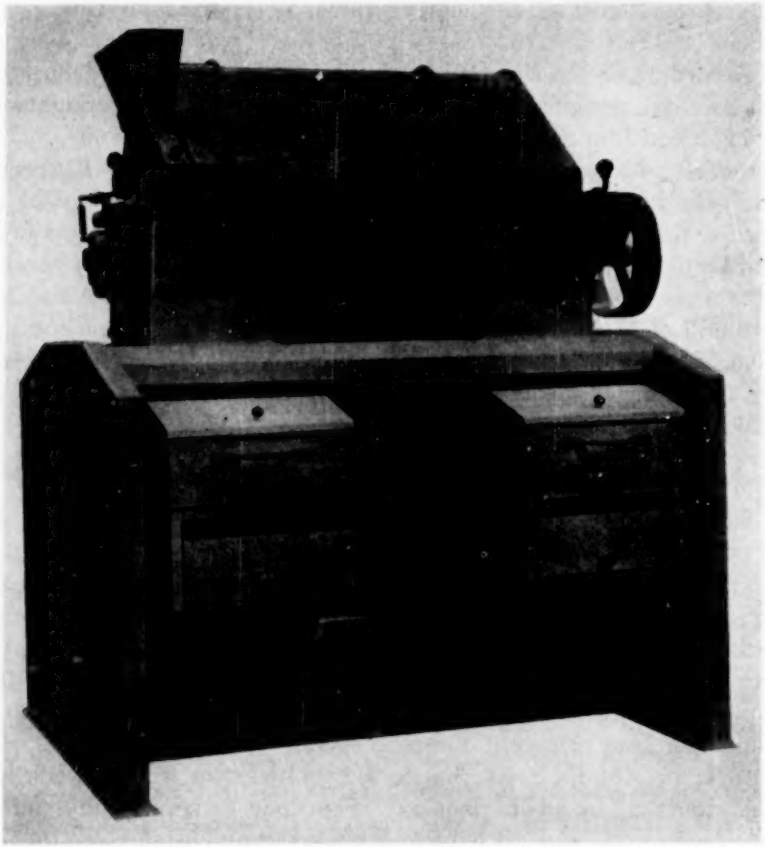

Fig. 1. The Buhler Mill.

of each stream in one test, as measured by the ferricyanide method of Blish and Sandstedt:

	Mg. maltose in 10 g.
First break	108
Second "	98
Third "	126
First reduction	186
Second "	235
Third "	270

The break flours are low in diastatic activity which corresponds to the findings of Leatherock, McGhee, and Giertz (1937) with streams of a commercial mill.

Table I gives some published figures illustrating the feature which the above named authors have drawn attention to, *i.e.*, that flours from experimental mills are deficient in diastatic activity as compared with

flours milled from the same wheat on a commercial mill. As a comparison a few figures are given of maltose contents obtained with the Buhler mill which prove that in laboratory milling flours can be produced with a normal diastatic activity. It is merely a question of the judicious choice of roll surface.

TABLE I

Investigators	Year	Mg. maltose: Commercial flour	Mg. maltose: Experimental flour	%
Markley and Bailey	1934	250	169	68
Swanson	1935	258	155	60
Pascoe *et al.*	1930	142	48	34
Sandstedt *et al.*	1937	250	140	56
Leatherock *et al.*	1937	229	148	65
Markley and Treloar	1937	226	150	67
		222	143	65
Buhler automatic mill—M.C.K.		198	200	101

Experimental

The introduction of the purifier was a mile-stone in the history of the milling industry and few millers today would care to dispense with this machine, which works with a high degree of efficiency if constructed along modern aerodynamic lines.

At first sight it would seem most natural to use purification in all test milling. It can, however, only be considered desirable in test milling if the purification of one or at the utmost two streams of middlings is found sufficient, since the test must become more foolproof, simpler and quicker than it is now. Sound milling experience on the other hand has brought out the necessity of carefully classifying the various stocks according to size before purification which has led to the use of a whole series of purifiers in a flow sheet.

Whereas Willard and Swanson (1911) gave no details of special tests with their experimental purifier, Scharnagel (1935) gave figures showing that 4.6 to 8.5% of stock was extracted by his purifier. The author decided to try the effect of a single purification first.

In the Buhler mill the spout conveying the middlings from the break sifter into the elevator leading to the first reduction was replaced by a purifier of simple construction which was thus fixed between the 2 sifters. The sieve was clothed with 60–50–40–24 GG silk. Care was taken to aspirate the whole surface and a brush was provided which automatically ran up and down under the bolting surface.

Canadian wheat grading No. 2 Northern, termed Manitoba 2 on the European market, was used for tests. The ash content of the

wheat was 1.57% (on a 13.5% moisture basis). The wheat was tempered to 13.5% and allowed to stand for several days in a closed vessel. Thirty minutes before milling, enough water was added to raise the moisture content to 15%. Three-pound samples were milled.

The rolls were set as follows:

	Roll distance in mm.
First break	0.52
Second "	0.12
Third "	0.10
First reduction	0.045
Second "	"
Third "	"

Following are the yield and ash figures of the break flours which were not analyzed separately in each test:

	% Yield	% Ash (13.5% moisture basis)
First break	5.5	0.65
Second "	8.5	0.51
Third "	2.0	0.69
Total break flour	16.0	0.58

The first result with the purification of the middlings was very encouraging.

MIDDLING TO FIRST REDUCTION

	% Ash
Without purifier	1.09
With purifier	0.51

An important decrease in the ash content of the straight-run flour was expected, or at least in the flours from the first and/or second reductions.

Tables II and III give the results of tests with and without a purifier. The production of break flour was constant in each series of tests with only a slight difference between the series which were not run at exactly the same time.

The "loss" is mostly around 2% when the mill is kept closed during the test. The high loss shown in Table III can be explained by the fact that samples often were drawn at different stages during the milling test to inspect the stocks and probably not enough attention was paid to the loss incurred.

The yield in sharps was reduced by 2.3 and 3% when purifying. The 3.7% and 3.6% extraction by the purifier went off mainly at the cost of the sharps. The purifier did not take away any flour, as shown by the yield of the latter and by the high ash figure of the purifier tails and filter product.

TABLE II

COMPARISON OF YIELD AND ASH CONTENT OF STREAMS OF THE BUHLER LABORATORY MILL WITH AND WITHOUT A PURIFIER (NO SCALPER UNDER THE FIRST REDUCTION ROLL)

	Without purifier		With purifier	
Flour	% Yield	% Ash	% Yield	% Ash
3 breaks	15.0	0.55	15.0	0.56
First reduction	30.0	0.39	32.2	0.44
Second "	16.0	0.54	14.3	0.54
Third "	5.6	0.81	4.0	0.89
Total flour	66.6	0.50	65.5	0.50
Sharps	9.6	2.42	7.3	2.46
Bran	20.6		20.6	
Purifier tails			2.3	2.67
" filter			1.4	1.66
Loss	3.2		2.9	
	100.0		100.0	

TABLE III

COMPARISON OF YIELD AND ASH CONTENT OF STREAMS OF THE BUHLER LABORATORY MILL WITH AND WITHOUT A PURIFIER (WITH A SCALPER UNDER THE FIRST REDUCTION ROLL)

	Without purifier		With purifier	
Flour	% Yield	% Ash	% Yield	% Ash
3 breaks	16.3	0.59	16.0	0.58
First reduction	32.0	0.40	32.0	0.40
Second "	11.3	0.48	12.3	0.50
Third "	5.7	0.80	5.3	0.94
Total flour	65.3	0.49	65.6	0.50
Sharps	11.0		8.0	
Bran	18.3		18.3	
Purifier tails			2.3	
" filter			1.3	
Loss	5.1		4.5	
	100.0		100.0	

Taking a mixture of first and second reduction flours, in Table II there is found 46% extraction with 0.442% ash without a purifier, as against 46.5% extraction with 0.450% ash with a purifier.

In Table III there is shown 43.3% extraction with 0.420% ash, as against 44.3% extraction with 0.429% ash.

As to the total straight flour there is no significant difference in the figures of either table.

Another system of purifier with special aspiration of the tails was tried. This would naturally mean a complication in the regulation of the air suction. The results were much the same as those given in Tables II and III.

Dividing the middlings into 2 streams according to size and purifying each separately was then tried but without obtaining an improvement which would warrant the use of any one of the systems of purifiers.

Summary

In general it is agreed that the experimental milling test is not yet satisfactory. The new Buhler automatic laboratory mill which is described permits greater uniformity, simpler handling and more rapid work than any other up to now. At the same time flour is produced with a diastatic activity similar to that obtained on a commercial mill, whereas many authorities give figures indicating that diastatic activity of experimentally milled flours is roughly only 65% of that of commercially milled flours.

In spite of the important part played by purification of middlings in commercial milling, very few applications of this principle seem to have been made so far in experimental milling. Figures are given which show that although an experimental purifier reduced the ash content of the middlings from 1.09% to 0.51%, the ash of the straight-run flour was not influenced nor was that of the combined first and second reduction flours. Neither did more elaborate purification help.

The author's results further emphasize the excellent work of eliminating bran particles done by a good sifter after the reduction rolls.

In the light of these results, it is not thought advisable to use a purifier in the experimental milling test as another variable would be introduced without a compensating improvement of the flour obtained.

Acknowledgment

Thanks are due A. Keller and F. Brassel for their help in carrying out this investigation.

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SUGARS IN BREAD

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(Read at Annual Meeting, May 1938)

It is probably natural for one who has spent much time in the production and use of sugar to question its function in any product wherein it seems essential. Shortly after my first introduction to scientific bread baking, I endeavored to find out just what happened to such sugars as were put into the dough but the information seemed absolutely unavailable. In 1935, analyses were made which indicated decided selection on the part of the yeast for certain sugars, a matter which has been discussed in the literature, but from which, however, no reliable conclusions could be reached because the authors did not note the differing action in various media; nor do I believe that reliable conclusions were possible, in as much as no methods for the accurate determination of combinations of sugars had been developed and published. Yeast must first invert cane sugar, and it is evident that one of the sugars resulting from the inversion is more adaptable than the other to the metabolism of the yeast. Recently, accurate analyses were made indicating the exact amounts of sugars remaining in baked bread, and while these analyses do not indicate that different relative amounts have not been present at certain stages in the process, nevertheless upon consideration of these results certain conclusions seem inescapable.

Table I indicates the formula used in producing bread—the only variable being the sugar used. Sweetened condensed milk, being the only milk product at hand, introduced a considerable amount of sucrose in all cases, as is indicated by the differentiation of the sucrose from the other milk solids in the table. The doughs were mixed in a small mixer, ten pounds of flour being used in each. Actual determination of the moisture was not made for all ingredients, the dry weights being based upon commonly accepted averages, and since the weight of all ingredients so closely approximate 100 pounds of dry material, the weights taken may be considered percentages within the limits of accuracy of the weighings made and the assumed moisture. The doughs were fermented for four hours with one punching and there was no noticeable difference in their action judged by the appearance of the baked bread.

TABLE I

STRAIGHT DOUGH FORMULA

(Dry weight of ingredients per 100 lbs. of normal flour)

Kind of sugar used	Sucrose I	Invert II	Hydrated dextrose III
Flour	88.0	88.0	88.0
Shortening	2.0	2.0	2.0
Yeast	0.9	0.9	0.9
Diastatic malt	0.8	0.8	0.8
Salt	2.0	2.0	2.0
Yeast food	0.25	0.25	0.25
Condensed milk solids—not sucrose	1.50	1.50	1.50
Condensed milk solids—sucrose	2.13	2.13	2.13
Sugar (anhydrous)	3.36	3.36	3.05
	100.94 lbs.	100.94 lbs.	100.63 lbs.

The first part of Table II is the result from calculating the total dextrose and levulose present in the dough on a dry basis. Water of crystallization has been considered moisture, and weights of dextrose and levulose have taken into account the increment due to inversion of sucrose when present. The second part of the table contains the sugars found in moisture-free bread baked from the doughs. The last

TABLE II

CALCULATED TOTAL OF ALL DEXTROSE AND LEVULOSE ADDED

Kind of sugar used	Sucrose I	Invert II	Hydrated dextrose III
Dextrose	2.89	2.80	4.17
Levulose	2.89	2.80	1.12
Analysis of Sugars in Baked Bread (Dry Basis)			
	I	II	III
Dextrose	0.18	0.17	0.55
Levulose	1.47	1.35	0.96
Maltose hydrate	4.56	4.52	4.24
Lactose	0.63	0.66	0.53
Sucrose	0.04	0.11	0.18
	6.88	6.81	6.46
Moisture in air-dried bread samples	10.46	10.04	9.20

line in this table shows the moisture percentages in the air-dried bread samples, which will be found to vary closely with the levulose present in the breads.

The methods used in making the analyses of the various sugars were described in a paper presented by F. W. Zerban and Louis Sattler before the Sugar Division of the American Chemical Society at Chapel Hill in April, 1937, and it is expected this paper will be published

shortly. The bread analyses were presented before the meeting of the American Chemical Society at Rochester in September, 1937, by the same authors and will also be published shortly. It will be noted that about 4% of sugar appears to have been consumed by the yeast in each one of the doughs, and that dextrose is the sugar to be consumed to the greatest extent, levulose the second, and maltose apparently the least. This selective action by the yeast seems more startling if we realize that the sugars must permeate the cell wall of the yeast where sucrose, after it has reached the inside, is inverted and the dextrose is consumed, while the levulose must certainly be excreted to be later reabsorbed when nearly all available dextrose has been consumed. Naturally small amounts of all sugars will remain unchanged outside the yeast cells because of lack of both physical contact and of sufficient osmotic pressure to permit of their passage through the cell wall. In order to make use of the maltose present, it is necessary that the yeast contain or provide certain enzymes to convert the maltose into dextrose. It is easily understood that the yeast will take advantage of the free dextrose present before exerting itself to the extent of the conversion of the maltose, and perhaps yeast which had been bred entirely on malt sugar would have shown somewhat different rates of assimilation when levulose and maltose were present; but yeast which has for generations been inverting sucrose as its primary food, while having a distinct preference for dextrose, will nevertheless consume levulose more rapidly than it will invert and ferment maltose.

There may be a time element required by the yeast in order to develop sufficient enzymes to provide itself with dextrose from inverted maltose, although this time element may not be generally noticed in gassing measurements because most flour contains sucrose or invert sugar in sufficient quantities to support the yeast for an appreciable length of time.

Table III indicates between 0.34 and 0.46% of levulose present in baked bread to which no sucrose or levulose had been added. This would indicate the original presence of nearly 1% of sucrose or its equivalent in invert sugar. Neither dextrose nor levulose occurs separately in nature; they are generally found in about equal quantities, and while dextrose is often termed "grape sugar" and levulose as "fruit sugar" the truth is that in grapes and fruits generally both sugars occur in equal amounts, and one is rarely, if ever, found without the other.

Perfection is very difficult of attainment in this uncertain world, and while the analyses in the cases of straight doughs seem so perfectly consistent and satisfying, the question was frequently raised as to what would happen in sponge doughs. To offer a satisfactory answer,

other loaves were made starting with the same sponge, which was divided into three portions, and to each portion was added five pounds of sugar: first, granulated sugar, second, a slightly modified granulated sugar, the modification being toward simulating the conditions in the mash from which the yeast was grown, and third, a hydrated dextrose. Analyses were made upon the air-dried crumb of the bread and calculated to dry basis as shown in Table III.

TABLE III

ANALYSES OF SUGARS IN BAKED BREAD

(Sponge-dough process)

	Sucrose	Modified sugar	Hydrated dextrose (A)	Hydrated dextrose (B)
Sucrose	—	—	—	0.06
Dextrose	0.31	0.00	2.24	3.01
Levulose	2.28	2.24	0.46	0.34
Maltose hydrate	3.97	4.45	1.76	1.04
Lactose hydrate	2.64	2.85	3.01	2.97
	9.20	9.54	7.47	7.42

It was immediately noticed that the total sugars remaining in the bread which was produced with dextrose (A) were about 2% less than in the sucrose breads, the difference being almost entirely in the amount of maltose present. Several weeks later, therefore, another baking test (B) was made in which dextrose was also used, and this checked the first test very closely as indicated in the fourth column of Table III. Gassing tests indicated that within the limits of experimental error the same amount of sugar was fermented in all these four breads. The analysis embraces a test for the combined sugars, and the sponge conditions being identical, there seems no explanation except that the large excess of dextrose or some substance which it contains has an inhibiting effect upon the diastase. In the last tests 0.33% of dry malt was used in the sponge (Table III), and as there is so much levulose found in the breads made, there must have been malt sugar produced in the sponge stage in sufficient quantity to support the yeast; this is borne out by the amount of malt sugar appearing in the breads made with cane sugar. After considerable study, it seems hardly possible that any of the other ingredients could have produced such unexpected results. While possibly the variations in the sugars in the first three breads shown in Table II are within the limits of experimental error, yet the difference between the total sugars in these cases show the same peculiar trend toward the reduced production of maltose in the presence of dextrose.

Aside from the adaptability of these various sugars as yeast food, consideration must be given to the question of which sugar is most desirable in the finished product and this question is made very pertinent now that it has been shown that dextrose may be eliminated and levulose allowed to remain.

It has been known for many years in sugar-refinery practice that levulose is broken down by heating much more readily than is dextrose, and this undoubtedly plays a large part in giving color to crust and to toast. It also is much the strongest retainer of moisture of all common sugars and its extreme sweetness, which is characteristic of honey, is quite familiar. It is a well-known fact that the majority of foodstuffs are rendered more acceptable to the average person by the addition of some sugar. Although nearly every food substance naturally contains sugar, its acceptance is generally increased by further additions of cane sugar. While there are possible exceptions to this statement, a great majority of persons appreciate a certain blend of sweetness with other flavors. In view of the present large additions of milk solids and shortening to bread, general acceptance by the consumer could undoubtedly be increased by raising the sweetness. By taking advantage of the information gained from these analyses just presented, sufficient cane sugar should be added to provide dextrose for the yeast both in sponge and dough stages—which, from the figures, would be about 4% of dextrose. This would leave 4% of levulose in the finished product, and because levulose retains at least half its own weight of moisture in a saturated atmosphere, such as in freshly baked bread, the levulose remaining, plus the moisture retained, would appreciably increase the weight of the salable bread produced. From the value of that increase, sucrose may easily be proved to be the cheapest source of sugar for yeast as well as the finest addition to flavor and the greatest preventive of staleness. Since it is apparently impossible to stop the retrogradation of the starch and the hardening of the gluten by simple commercial means, the best protection for bread against staleness is the maintaining of softness by the shortening and other ingredients and the retention of moisture by the sugar and the partially converted starch, the moisture retention of which considerably exceeds that of maltose. Since the conversion of starch into a sweet hygroscopic sugar is limited in the preparation of bread to the small percentage of starch grains which are ruptured, it is quite essential to add other sweet moisture-retaining substances for this purpose to the dough. Dextrose is of little use for this purpose since it retains less than 25% as much moisture as levulose, it has only about one-third the sweetening power, its sweetness has not the same flavor as levulose or sucrose, and it is generally not considered as palatable.

Besides, dextrose is the first sugar to be fermented, but little is left at the finish in the baked bread, where, as has been indicated, its presence would serve no useful purpose, in any case, either as a sweetening agent or as a moisture-retainer.

Conclusion

Recently developed analytical methods have made possible the accurate determination of the sugars which remain in baked bread. The analyses show that the yeast prefers as its food substance dextrose in preference to the other sugars which may be present, and by proper adjustment of quantities, the final product may contain sufficient levulose to be of value as a moisture retaining and high sweetening agent. Some as yet unexplained results occur in bread baked with dextrose as the only added sugar. In such loaves, the total of all sugars present is appreciably less.

Acknowledgment

I wish to express my great appreciation for the very considerable assistance rendered by a large number of friends of this Association. Without their aid and counsel, this paper would not have been possible.

OXIDATION OF STARCH—ACTION OF BROMINE ON GELATINIZED CORN STARCH [1]

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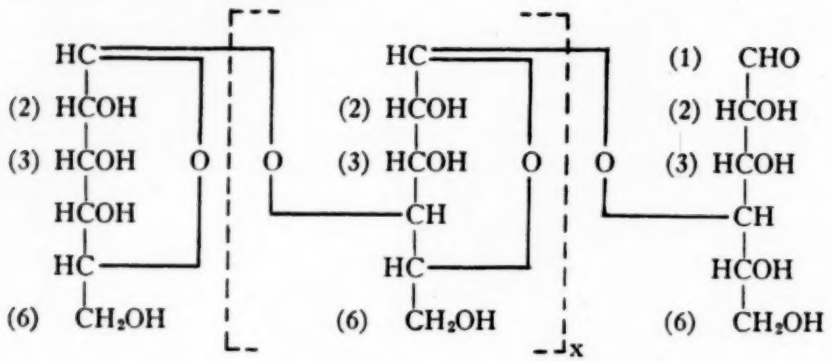
(Received for publication March 1938)

Oxidation is used to some extent in the technical production of modified starches. The literature on the reaction is limited mostly to process patents for producing modified starches or to descriptions of the change in physical properties of starch when subjected to various oxidizing agents. A general summary of the earlier literature is given by Samec (1927) and a bibliography by Walton (1928).

Busch and co-authors (1936) have summarized the literature on the oxidation of simple sugars. The available information suggests four different types of oxidation for starch:



Type I—Oxidation of aldehyde groups to carboxyl groups (at 1).

Type II—Oxidation of primary alcohol groups to carboxyl groups (at 6).

Type III—Oxidation of secondary alcohol groups to ketone groups (at 2 or 3).

Type IV—Oxidation of glycol groups (at 2 and 3) to carboxyl groups with concomitant rupture of the pyranose ring.

The oxidation of starch was concluded to be of the first type by Samec (1929, 1933) and Samec and Blinc (1933). They claim that the formation of acid groups results from the oxidation of aldehyde groups.

[1] Journal Paper No. J-369 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 426. Supported in part by a grant from the Corn Industries Research Foundation.

Their observations were made on the mild oxidation of starch by ammonium persulfate, sodium perborate, potassium permanganate, sodium peroxide, hydrogen peroxide, and chlorine.

An earlier investigation by Syniewski (1925) suggested a reaction of the second type, in which one-third of the glucose units were oxidized at the primary alcohol group rather than at the aldehyde group. He oxidized amylodextrin with bromine, maintaining a neutral solution with barium carbonate, and based his conclusions on the furfural obtained when the oxidized product was distilled with hydrochloric acid. The work of Kalb and Falkenhausen (1927), and of Ritter and Seborg (1929) on cellulose oxidation is in agreement with the conclusion of Syniewski.

Secondary alcohol oxidation of sugars was effected by Reichstein and Neracher (1935), Cook and Major (1935), and Everett and Sheppard (1936). Everett and Sheppard also report secondary alcohol oxidation of starch. The products of these oxidations possessed reducing properties due to ketone groups.

The oxidation of a glycol grouping (Type IV) has been reported by Jackson and Hudson (1936). They found that the pyranose ring of the sugar derivative undergoes rupture at the glycol group and forms two carboxyl groups.

Although the last type of oxidation was reported only on a simple sugar derivative and the third type chiefly on simple sugars rather than on starch, the glucosidic structure of starch would indicate such analogies between starch oxidation and that of the simple sugars.

It is apparent that three different interpretations of starch oxidation have been presented in the literature. The conclusion of Samec that the carboxyl groups result from aldehyde groups is not compatible with that of Syniewski, which traces their origin to primary alcohol groups. On the other hand, Everett and Sheppard criticize Syniewski's conclusion and present a third explanation. The correct interpretation can be determined only by hydrolysis of the oxidized starch and subsequent identification of the hydrolytic products. The purpose of the present study was to follow the course of the oxidation reaction by analytical methods in order to establish conditions of oxidation best suited for the preparation of oxidized starches for hydrolysis.

The procedure of Dickson, Otterson, and Link (1930) for uronic acid determination was used to follow the primary alcohol oxidation of starch. The uronic acid is determined by heating the sample with 12% hydrochloric acid and measuring the carbon dioxide evolved. It should be recognized that under these conditions carbon dioxide

might be obtained from structures such as keto-acids, which are not strictly glucuronic acid units.

Secondary alcohol oxidation to ketone groups was measured by Fehling's solution and reported in terms of milligrams of copper per hundred milligrams of oxidized starch. Under the present conditions of oxidation it is not conceivable that these products could contain any reducing groups other than ketone groups.

The carboxyl groups resulting from aldehyde oxidation could be estimated by means of the calcium content in excess of that needed for uronic acid carboxyls. The measure of these was an indication of the extent of rupture of the starch chain and of glycol oxidation.

Bromine was chosen as the oxidizing agent, since it would simulate the sodium hypochlorite oxidation used commercially and yet be conveniently handled in the laboratory. In the presence of calcium carbonate the pH of such an oxidation remains at the neutral point and degradation of the starch molecule by both acid hydrolysis and glycol splitting would be expected to remain at a minimum.

Preparation of Soluble Calcium Salts of Oxidized Starch

A cold water suspension of twenty grams of starch was poured into 500 c.c. of boiling water. The resulting solution was boiled gently for thirty minutes. An excess of calcium carbonate was then added, the amount being the same as the weight of the bromine used, viz., 5, 10, 20, 30, 40, and 60 grams for ½, 1, 2, 3, 4, and 6 bromine equivalents per glucose anhydride unit. The thick mixture of starch and calcium carbonate was cooled to room temperature, placed in a three-necked flask, and the bromine then added slowly while the reaction mixture was being stirred mechanically. Soon after the addition of the first few drops of bromine the thick paste became quite fluid. The solution remained neutral to litmus throughout the reaction. No violent oxidation or appreciable rise in temperature occurred. Using ½, 1, 2, 3, 4, and 6 bromine equivalents per glucose, the reaction was allowed to continue for ½, 1, 1½, 2, 2, and 3 days respectively. The excess bromine was removed by aeration. The residual calcium carbonate was filtered off and the slightly turbid filtrate poured into twice its volume of 95% alcohol. The white flocculent precipitate was allowed to settle for a few hours, then filtered with suction and washed with alcohol. Samples for analysis were repeatedly dissolved in water and poured into a three-fold volume of 95% alcohol until the filtered precipitate showed no halogen test. After a sodium fusion the test was still negative. These oxidized starch samples, with the exception of the sample oxidized by ½ bromine equivalent, gave no coloration with iodine solution. Yields are summarized in Table I.

Preparation of Insoluble Calcium Salts of Oxidized Starch

With 6, 8, and 10 bromine equivalents per glucose unit, the oxidized starch yielded a considerable amount of insoluble calcium salt. The oxidation procedure was identical with that for the soluble calcium salts. Three, four, and six days were required for the oxidation by means of 6, 8, and 10 bromine equivalents respectively. The residue from the oxidation was filtered off and treated to remove calcium carbonate, as outlined below. Clear and colorless filtrates resulted, whereas in the preparation of the soluble calcium salts they were turbid. The filtrate from the oxidation by ten equivalents of bromine showed only a trace of soluble calcium salt which was therefore not recovered. The soluble calcium salt from the other oxidations (using 6 and 8 bromine equivalents) was recovered.

The insoluble residue from the oxidation was dried and ground to a very fine powder. This was suspended in an equal volume of water and titrated with dilute hydrochloric acid (1 : 1) while being stirred mechanically. When effervescence ceased, the titration became very slow and was followed to pH 4–5, with sodium alizarin sulfonate used as an outside indicator. The resulting suspension was neutralized to litmus with sodium hydroxide. The precipitated calcium salt of the oxidized carbohydrate was filtered off and washed with dilute alcohol till free from halides. The calcium salt gave no test for carbonate. The yields are presented in Table I.

TABLE I

YIELDS OF OXIDIZED STARCH FROM 20 GRAMS OF CORN STARCH CONTAINING 10.5% MOISTURE

Bromine used	Reaction time	Yield of soluble Ca salt	Yield of insoluble Ca salt	Total yield of oxidized carbohydrate Ca salt less calcium
Grams	*Days*	*Grams*	*Grams*	*Grams*
5	½	15	0	14.9
10	1	17	0	16.8
20	1½	18	0	17.5
30	2	18	0	17.2
40	2	18	0	16.8
60	3	9	*	*
60	3	3	16	16.9
80	4	2	17	16.6
100	6	0	23	19.6

* Not determined.

Analytical Comparison of Oxidized Starches

The purified oxidized starch samples were analyzed for calcium, reducing power, and uronic acid content. The specific rotations were

measured on all samples, the insoluble calcium salts being dissolved in a small excess of N/10 hydrochloric acid. See Table II.

TABLE II

ANALYSIS OF CALCIUM SALTS FROM OXIDIZED STARCH

Bromine used	Calcium	Reducing value	Uronic acid equivalent on pure carbohydrate	Rotation in water
Equivs. per glucose	%	*Mg. Cu per 100 mg.*	%	$[\alpha]_D^{25}$
½	0.53	24.5	10.8	+182.8°
1	1.14	39.3	19.7	+181.8°
2	3.01	46.2	38.4	+171.0°
3	4.43	39.5	43.7	+148.9°
4	6.40	37.3	47.5	+113.6°
6	7.43	40.2	50.7	+ 90.8°
6*	11.04	15.6	49.2	+ 75.7°
8*	12.83	18.1	48.0	+ 73.9°
10*	14.81	15.2	32.1	+ 47.3°
Syniewski's oxidized starch (1925)		28.4	33.9–34.5	+191.1°

* Insoluble calcium salt.

The calculation of the uronic acid content was made on the basis of the percent of glucuronic acid anhydride present in the sample after the weight of the calcium present had been subtracted. Table III

TABLE III

COMPARISON OF CALCIUM FOUND AND CALCIUM REQUIRED TO NEUTRALIZE THE CALCULATED URONIC ACID CONTENT OF OXIDIZED STARCH

Bromine per glucose	Uronic acid equivalent	Calc'd Ca to neutralize uronic acid	Calcium found	Difference
Equivs.	%	%	%	%
½	10.8	1.23	0.53	−0.70
1	19.7	2.13	1.15	−0.98
2	38.4	4.36	3.10	−1.26
3	43.7	4.97	4.64	−0.33
4	47.5	5.39	6.84	+1.45
6	50.7	5.76	8.03	+2.27
6*	49.2	5.59	12.42	+6.83
8*	48.0	5.46	14.73	+9.27
10*	32.1	3.64	17.39	+13.75

* Insoluble calcium salt.

compares the amount of calcium found and the amount calculated to neutralize the uronic acid.

The data of Table III are illustrated in Figure 1. The curve showing the difference between the observed calcium content and the calcium required for the uronic acid is indicative of the production

of non-uronic carboxyls from aldehyde and glycol groups. Since these must be formed by hydrolysis or oxidative rupture, the curve indicates that neither of these two types of cleavage could be appreciable until more than three bromine equivalents were consumed. The relation between the degree of oxidation and the amount of calcium calculated to neutralize the uronic acid (Fig. 1) shows the uronic acid production passing through a maximum when six equivalents of bromine have been absorbed.

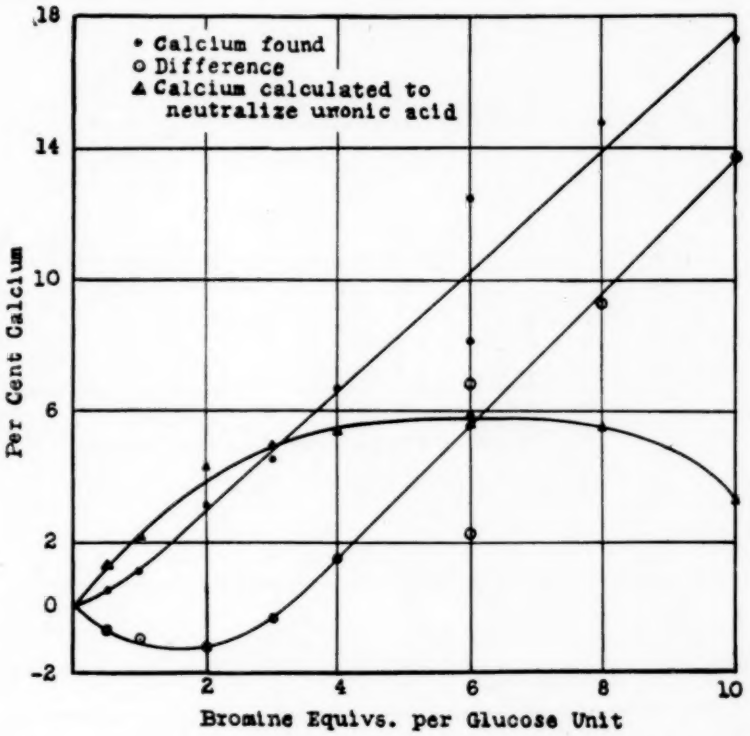


Fig. 1. Relationship between the calcium found in starch oxidized to varying degrees and the calcium calculated to neutralize the uronic acids present.

Some insight into molecular size is given by the optical rotatory power. The molecular degradation in view of this measurement follows the course indicated by Figure 2. The relation between the optical rotatory power and the uronic acid content is shown in Figure 3. The relation between the reducing value and the equivalents of bromine is presented in Figure 4. The decline of the curve when more than two bromine equivalents were used represents the decomposition of the reducing units, whether these be keto acids or glucose anhydride

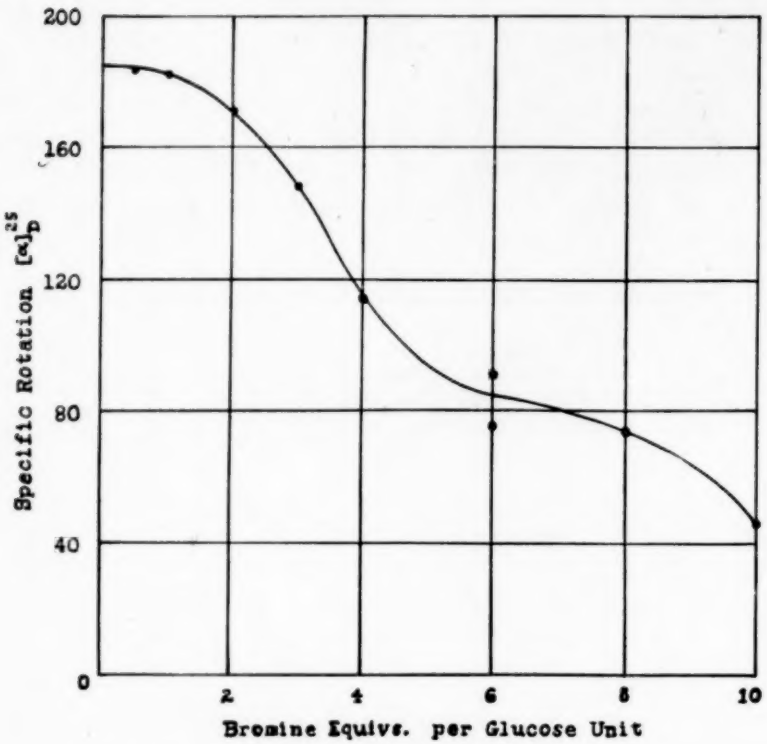


Fig. 2. Decrease in optical rotatory power of oxidized starch products.

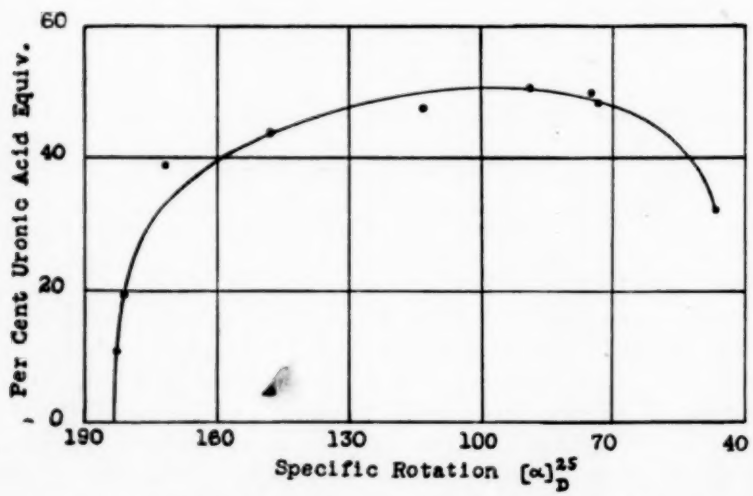


Fig. 3. Relationship between uronic acid content and optical rotatory power.

units containing keto groups. The beginning of this effect coincides with the beginning of rupture by glycol splitting and hydrolysis (Fig. 1). This seems to argue for the precedence of glycol spitting rather than hydrolysis. Figure 4 resembles Figure 2 in that beyond six equivalents of bromine both curves show a break in the direction of zero slope.

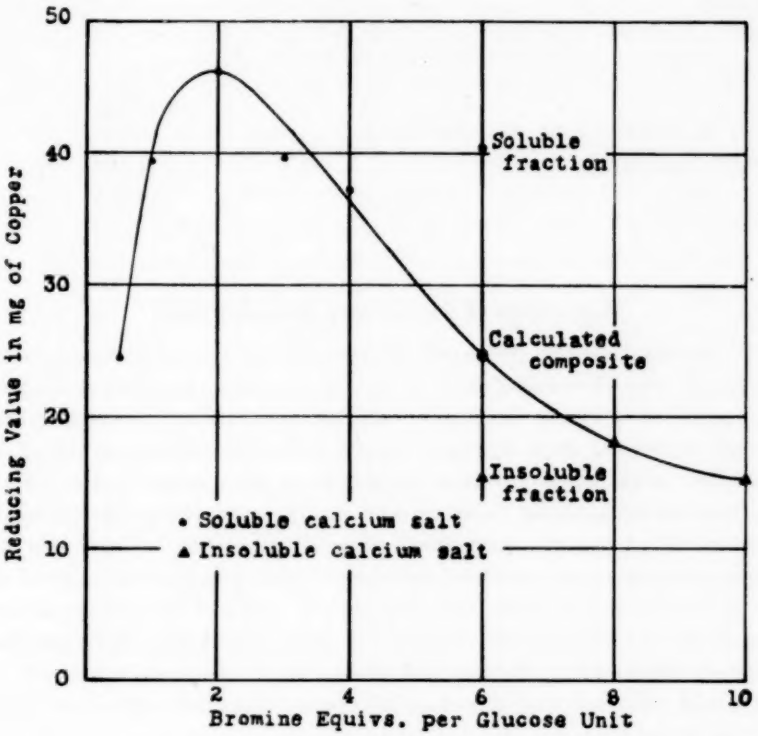


Fig. 4. Variation of reducing value with extent of oxidation.

Preparation of the Free Oxidized Starch Acids

In order to obtain the oxidized starch free from calcium, the salt was treated with an excess of oxalic acid, the calcium oxalate filtered off, and the carbohydrate precipitated by pouring into 95% alcohol. The precipitate was dissolved in water and again poured into 95% alcohol. This procedure was repeated until the solid gave negative tests for both oxalic acid and calcium. The free acids were much more soluble in alcohol than the calcium salts and there was considerable loss of material in the preparation. The free acids were very gummy precipitates in contrast to the flocculent ones obtained with the calcium

salts. From the products obtained by the oxidation of starch using six bromine equivalents, no free acid that was insoluble in alcohol was secured. This observation, together with the known loss in the above preparations, indicates that a fractionation of the free acids took place on purification. The free acids were analyzed for uronic acid equivalent and their rotations measured with the results as shown in Table IV.

TABLE IV

ANALYSIS OF OXIDIZED STARCH ACIDS

Bromine	Uronic acid equivalent	Rotation in water
Equivs. per glucose	%	$[\alpha]_D^{24}$
2	33.68	+172.5°
3	40.62	+171.6°
4	43.77	+151.6°

Oxidation of Alpha- and Beta-Amylose

A modified starch prepared by twenty hours of the Gore (1928) treatment was electrodialyzed to obtain a soluble fraction resembling beta-amylose and an insoluble fraction resembling alpha-amylose. Twenty grams of each fraction were oxidized according to the above procedure with three bromine equivalents per glucose unit. There was greater agreement between the analyses of these two products from modified starch than there was when either of the two was compared with other oxidized starches. This was true in spite of the fact that the beta-amylose was completely oxidized in half the normal time that was needed for gelatinized starch, and the alpha-amylose required almost twice the normal time. In the face of such evidence, one would conclude that the chief differences between alpha- and beta-amylose were physical and not chemical.

Discussion of Results

The evidence indicates that the four types of starch oxidation which would be expected from theoretical considerations take place when starch is oxidized by bromine in neutral solution.

The uronic acid determination used to measure the oxidation of primary alcohol groups indicates that the maximum production of uronic acid is reached when six equivalents of bromine per glucose unit are used, this maximum corresponding to 50.7% uronic acid in the starch. This means that approximately half of the primary alcohol groups were oxidized to uronic carboxyls, either glucuronic acids or keturonic acids. Keturonic acids would not be expected to produce

furfural and their presence can be inferred from the fact that a furfural determination indicated about two-thirds as much uronic acid as was obtained by the carbon dioxide evolution method.

Glucose, untreated starch, and commercial, alkaline hypochlorite-treated starch gave approximately 1.5% uronic acid as a blank correction for the determination. It was concluded, therefore, that no appreciable amount of carbon dioxide evolved in the determination of uronic acid came from unoxidized carbohydrate. The soluble oxidized fraction is so completely removed from the commercial alkaline hypochlorite-treated starches that they give no greater value for uronic acid than untreated starch.

Oxidation of other portions of the starch molecule to produce carboxyl groups which were non-uronic began to occur when more than three equivalents of bromine were used and became a very significant factor as the amount of bromine was increased. With the use of more than six equivalents of bromine these non-uronic carboxyl groups constitute the major portion of the total carboxyl content. As oxidation proceeded at other portions of the glucose chain, the terminal carboxyl group would also become non-uronic in character. Glycol splitting and rupture of the starch chain have taken place. Both the decrease in rotation and the increase in calcium content were in agreement with molecular degradation of the starch molecule.

The reducing value of the oxidized starch products showed that the secondary alcohol oxidation quickly reached a maximum value with two bromine equivalents. The decrease in reducing power beyond this point has been interpreted as due to further oxidation of the reducing units.

As the oxidation of starch proceeded the products became progressively less soluble. Insoluble calcium salts of the oxidized starch were first noticed when six bromine equivalents were employed, whereas further oxidation produced practically all insoluble material.

The oxidation of alpha-amylose followed the same course as the oxidation of beta-amylose. A soluble product was formed from the insoluble granule covering. Microscopic observations indicate that commercial oxidations of raw starch consist primarily in an attack on the granule covering. The envelope of the starch granule is disintegrated by oxidation and removed by washing, thus leaving a starch which gives a less turbid paste than raw starch.

Summary

Starch was oxidized by varying amounts of bromine in the presence of calcium carbonate. The oxidized carbohydrate was recovered as the calcium salt of the acid. These calcium salts were analyzed for

uronic acid, calcium, and reducing power, and the specific rotations were measured.

An analytical comparison of the oxidized starch products indicates that the four expected types of oxidations have occurred:

1. Oxidation of primary alcohol groups as estimated by the carbon dioxide evolution method reached a maximum when six equivalents of bromine per glucose unit were used. The values obtained indicated 50.7% when calculated as glucuronic acid anhydride. The evidence indicated that a portion of this carbon dioxide came from keturonic acid structures rather than from glucuronic acids.

2. Oxidative production of non-uronic carboxyl groups was measured by the calcium content in excess of that calculated to neutralize the uronic acids present. Non-uronic carboxyls were not produced in appreciable quantity until more than three equivalents of bromine were used.

3. Secondary alcohol oxidation to ketone groups as measured by reducing value reached a maximum value with two bromine equivalents.

4. Oxidation of glycol groups was indicated by the decomposition of the reducing units in the late stages of oxidation, and by the molecular degradation of the starch molecule as established by the decrease in optical rotatory power and the increase in calcium content.

Some of the calcium salts were converted into the free acids of the oxidized carbohydrate.

The oxidation of alpha-amylose followed the same course as that of beta-amylose, but the beta-amylose required one-fourth as much time as the alpha-amylose.

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A STUDY OF THE EFFECT OF PROTEOCLASTIC ENZYMES UPON WHEAT GLUTEN SOLUBILITY IN SODIUM SALICYLATE SOLUTIONS

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(Received for publication June 4, 1938)

The importance of proteoclastic action from the standpoint of the baker and consumer is now widely recognized. The harmful effects of too high a proteolytic activity in fermenting doughs are well known. This results in weak, runny doughs which are incapable of retaining the gas formed during fermentation and produce flat, badly shaped loaves of poor texture and appearance. On the other hand, doughs which have harsh, "bucky" glutens may be improved by the addition of appropriate quantities of proteolytic enzymes. One of the beneficial effects of malt syrups and malt upon some types of high-gluten wheat-flour doughs appears to be due to the increased proteolytic activity conferred by the malt enzymes which soften and ameliorate the condition of the gluten.

The problem of activation and repression of proteolytic enzymes is of direct practical interest. The use of glutathione, in one form or another, as a flour protease activator has become quite common, while potassium bromate in small increments may function as a repressor of proteolytic activity, and in this way the favorable responses in baking obtained by its use might be explained. It is also possible that the beneficial effect of bromate may be due in part at least to some action of the chemical upon the flour gluten itself. Further experimental investigation of proteoclastic enzymes and the mechanism of this action in wheat flour doughs is needed.

Review of Literature

The action of various proteolytic agents has been extensively studied and a number of papers have recently appeared in the literature dealing with their effect upon gluten properties and baking characteristics. Proteases of wheat flour were investigated by Ford and Guthrie (1908). A mixture of flour and 1.5% gelatin solution caused a liquefaction of the gelatin. An aqueous flour extract had similar effects upon gelatin. These researchers also observed that the addition of active proteases to bread dough caused a reduction in tenacity and a

modification of gluten properties. Their investigations definitely established the presence of proteases in wheat flour. Baker and Hulton (1908) corroborated the evidence of Ford and Guthrie. Flour extract altered the appearance of egg albumin, although no increase in soluble nitrogen was detected.

The Sorenson formol titration was used by Swanson and Tague (1916) to measure increases of amino-nitrogen in flour extract, but only a relatively small change was observed during a four-week period. Stockham (1920) found that the lower the flour grade the shorter the time required to liquefy 1.5% gelatin jelly. It was also found that the germ end of the kernel was more active than the blossom end. Sharp and Elmer (1924) postulated an increase in amino-nitrogen, as well as 5% potassium sulfate soluble nitrogen, with the autolytic digestion of flour, and considered that the flour "gliadin" was readily attacked by flour proteases.

Olsen and Bailey (1925) concluded that the properties of gluten were not appreciably affected by proteases contributed by baker's yeast during a 4–5 hour fermentation period. These workers attributed changes in water-imbibing capacity of the gluten to alterations in the pH of the medium.

Cairns and Bailey (1928) studied the utility of eight chemical methods for evaluating the progressive proteolysis in flour suspensions. The formol titration method of Sorensen appeared to be best suited to the measurement of proteolysis in flour. The amount of proteolysis as measured by the appearance of amino-nitrogen was found to be small. The presence of proteases in flour was demonstrated. Tissue and Bailey (1931) thought that little proteolysis took place in flour suspensions made from hard wheat flours, and also found Sorensen's method the more satisfactory for measuring proteolysis in flour suspension.

Definite improvements in "bucky" doughs produced by the action of proteolytic enzymes were noted by Read and Haas (1934). Yeast autolysate also improved the qualities of dough, while its saccharifying activity was practically negligible. Benefits resulted from the use of papain, pepsin, and trypsin in decreasing degree. Later, Read and Haas (1936) confirmed these conclusions in a further study of dough improvement through addition of various proteolytic agents. These workers further showed (1937) that excessive quantities of proteinase or proteinase activator will ruin the baking properties of any gluten. In a later publication (1937a) the repression of proteolysis by oxidizing agents, notably potassium bromate, was discussed. Repression of the activity of papain and bromelin by relatively large quantities of bromate were indicated.

Balls and Hale (1936) postulated that in the course of flour-dough fermentation the action of proteinase changed the colloidal properties of the wheat protein, this change finally becoming evident in the properties of the gluten. The first change would be to form a more tenacious gluten which would become thinner and more liquid upon the further addition of proteinase. A small quantity of the enzyme would therefore be beneficial, while a large amount would be very harmful. The improvement of flours upon bleaching or exposure to oxygen was attributed by these workers to the oxidation of an activator of the flour proteinase. A similar effect would be obtained upon the addition of an oxidizing agent to bread doughs.

Jørgenson (1935, 1936) investigated the properties of glutathione in relation to gluten strength. This substance is present in yeast, as shown by Hopkins (1921). Later its constitution was proved by Hopkins (1929) to be a tripeptide containing glycine, glutanic acid, and cysteine. Glutathione possesses no proteolytic activity itself, but functions as an activator upon the native proteases of the flour. Jørgenson concluded as a result of this investigation that flour contained powerful but latent proteolytic enzymes which when activated by glutathione may ruin the gluten of bread dough, resulting in the production of undesirable loaves. Balls and Hale (1936) concluded that flour proteases can be activated by glutathione or by cysteine; a relatively large dose of the latter would also attack the protein itself. Sullivan, Howe, and Schmalz (1936) isolated glutathione from wheat germ and showed its harmful effect upon gluten. Later the same workers (1937) concluded that oxidation of glutathione was in part responsible for the beneficial effect of heat treatment on wheat germ and flour streams containing germ. Flohil (1936) studied the action of various oxidizing agents and flour improvers upon papain and flour protease and concluded that these chemicals reduced proteolytic activity through oxidation, thus explaining the beneficial effect of these agents upon flour dough. Kozmin (1934) postulated that the principal action of "improving agents," as bromate and iodate, permanganate, etc., is to produce coagulation of the gluten.

Bohn and Bailey (1937) found that papain preparation significantly decreased stress readings when added to dough in appreciable quantities. These researchers stated that their finding agreed with the theory that small additions of papainase acted as a coagulant upon wheat protein, whereas larger amounts exerted a liquefying action. Landis (1935) and Rupp and Bailey (1937) substantiated the degrading effect of papain upon flour dough gluten by means of the farinograph. Pepsin differed in its degrading action, and the comparative effects produced on the gluten of one flour by the two enzymes were not

necessarily the same as they were when the same enzymes acted upon the gluten of other flours.

Jørgenson (1935) discovered that ascorbic acid could be used as a bread improver in much the same way as potassium bromate. Further studies regarding this substance were carried out and reported by the Wheat Research Institute, Christchurch, New Zealand (1937). It was found that lemon juice gave satisfactory results as a substitute for bromate as the improver. Melville and Shattock (1938) proved that dehydroascorbic acid, formed by the oxidation of ascorbic acid, functions as efficiently as bromate on a weight for weight basis. These workers found that both these acids inhibit papain, the dehydroascorbic acid being a stronger inhibitor than the ascorbic acid.

Experimental Material and Methods

A study of literature citations in the field of proteoclastic enzymes with reference to their influence upon flour gluten and baking quality indicated the desirability of obtaining data relative to the effect of these enzymes upon the solubility of gluten protein in sodium salicylate solution. Solutions of sodium salicylate have been shown to have little denaturing action upon flour gluten by Cook and Alsberg (1931), Cook and Rose (1934, 1935), and Harris (1937, 1938). It has also been demonstrated by Sinclair and McCalla (1937) that gluten solubility in sodium salicylate decreases with baking strength when dealing with flours injured by storage. It would be logical to expect that a change in the aggregation of the gluten protein complex brought about by enzymic action would be reflected in the rate at which the washed gluten dispersed in sodium salicylate. If such an effect were demonstrated, the influence of increments of potassium bromate upon proteolytic action, a problem which has been the subject of debate among cereal technologists, could also be investigated from this angle. In order to obtain information on these points the following investigation was undertaken.

A sample of commercial patent hard red spring-wheat flour, of which a relatively large quantity was available, was selected for this study. This flour had been milled from the 1936 crop and contained 13.1% of protein and 0.40% ash. It had been bleached in the customary manner.

Preliminary trials were made with the method used by Flohil (1936) in which a quantity of washed gluten was cut into small pieces and placed in a 100 c.c. test tube with 50 c.c. of distilled water plus a few drops of boric acid to inhibit bacterial action. The proteolytic agent was then added, the tubes were thoroughly shaken, and stoppered with absorbent cotton. The tubes were then placed in a controlled fer-

mentation cabinet at 30° C. for 36 hours until disintegration of the gluten appeared to have taken place. Trouble was experienced with bacterial and fermentative action, however, and the gluten, which was recovered with difficulty, failed to yield satisfactory results when dispersed in sodium salicylate solution. In some cases it was found quite impossible to recover sufficient gluten to place in solution. Toluene was also tried as a preservative, but it was not entirely satisfactory and in addition it is known to possess an inhibitory action upon the enzyme.

After a few initial trials a satisfactory method was developed which had the merit of using gluten washed from doughs made up in the usual manner for baking purposes. In this procedure 50 grams of flour were mixed for 3 minutes in the Hobart-Swanson mixer to a dough of standard consistency (in the case of heavy enzyme treatments it was found necessary to reduce the water added) containing the standard ingredients flour, distilled water, yeast, sugar, and salt in the proportions used for baking in this laboratory. The enzyme preparations were used in the form of freshly prepared solutions or suspensions. The glutens were washed immediately from the doughs under a small standardized stream of 0.1% sodium phosphate solution of pH approximately 6.8–7.0, as recommended by Dill and Alsberg (1924), and used by the author in previous fractionation work. Approximately 10–12 minutes were required to wash the gluten, which was then allowed to stand immersed in the phosphate solution for at least 15 minutes before weighing and dispersion. For these dispersions, 18 grams of gluten were cut into small pieces as uniformly as possible and placed in 300 c.c. of 10% sodium salicylate solution contained in 500 c.c. Erlenmeyer flasks. The flasks were vigorously shaken immediately following the addition of the gluten, and at short intervals during the first 12 hours of the dispersion period. During the remaining 24 hours of dispersion the flasks were shaken only occasionally, as the larger portion of the gluten had been dispersed and the rate of dispersion was relatively slow. A few drops of capryl alcohol were added to each flask from time to time to prevent excessive foaming. Aliquots of the liquid were taken off at suitable intervals, centrifuged to remove any particles of undispersed gluten and suspended starch, and 10 or 5 c.c. duplicates, depending upon the length of time elapsed after dispersion commenced, were pipetted off for nitrogen determinations by a modified Kjeldahl method.

A number of proteolytic enzymes from both the animal and vegetable sources were selected for investigation. Yeast water was also included since it is a convenient source of glutathione, which functions as a flour protease activator. This yeast water was prepared according to

the procedure used by Jørgenson (1936) and consisted in boiling a mixture of equal quantities of pulverized yeast and water for a 10-minute period. The mixture was cooled, centrifuged, and the supernatant liquid decanted. The yeast water was always freshly prepared to obviate danger of oxidation of the glutathione with consequent loss of activating power. The heating would necessarily inactivate the yeast enzymes and any proteoclastic activity observed in using the preparation would accordingly be due to activation of the flour proteases themselves.

It was noticed that the glutens washed from doughs containing relatively high concentrations of proteoclastic enzymes dispersed much more rapidly than was the case where the glutens were washed from dough prepared without added enzyme, while gluten from dough containing a small enzyme concentration was intermediate in respect to speed of dispersion. The solutions in which these treated doughs were placed became rapidly opaque and the gluten speedily lost its coherence and characteristic appearance, becoming distributed throughout the solution. The control glutens, on the other hand, remained intact for hours before showing signs of dispersal, but they dispersed more rapidly, nevertheless, than gluten washed from doughs made without yeast. This difference was doubtless caused by the presence of proteolytic enzymes and glutathione contained in the yeast itself. The addition of small quantities of potassium bromate to the dough appeared to delay the dispersion of the gluten in some instances, especially when the concentration of enzyme was relatively high. Bromate, however, never retarded the dispersal of the treated doughs to the same degree as the untreated, control dough. Bromate appeared to increase the difficulty of washing the gluten from some of the treated doughs, notably when the treatment consisted of addition of taka diastase and bromelin. When yeast water was used bromate appeared to "bind" or stiffen the dough, and increased the ease of washing. It seemed that this effect was not entirely due to the effect of enzyme inactivation.

Discussion of Results

In Table I are shown the data obtained from various enzyme treatments upon the dispersion of the glutens washed from a series of doughs. The results are expressed as milligrams of soluble protein in 100 c.c. of dispersion and cover a dispersion period of 36 hours. As the dispersion time was prolonged the time intervals between samplings were progressively increased. Frequent sampling over the entire 36-hour period appeared unnecessary and would have required the preparation of excessively large quantities of the dispersions.

TABLE I

EFFECT OF VARYING CONCENTRATIONS OF PROTEOLYTIC ENZYMES UPON THE QUANTITY OF WHEAT GLUTEN WASHED FROM DOUGHS AND DISPERSED IN SODIUM SALICYLATE

Results in Milligrams of Protein per 100 c.c. Dispersion

Treatment	Time of dispersion (hours)						
	1	2	4	8	12	24	36
	mgs.	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*
Control	118	152	257	506	702	1453	1524
Papain, 0.002%	101	168	239	413	818	2223	2306
0.004%	215	226	362	1419	1799	——	2138
0.008%	142	142	234	855	1756	2480	2542
0.012%	139	299	1134	2151	2211	2226	2222
0.020%	463	1274	1465	1576	1608	1630	1636
Yeast water, 10 c.c.	139	245	576	1385	1600	1796	1790
20 c.c.	205	533	903	1547	1608	1618	1620
30 c.c.	467	872	946	1439	1436	1436	1440
Pepsin, 0.1%	111	168	235	564	866	1784	——
0.4%	701	1077	1273	1458	1482	1494	——
Pancreatin, 0.01%	248	775	1274	1619	——	——	——
0.03%	1308	1214	1333	1328	——	——	——
Malt diastase, 0.5%	131	311	1351	1379	1678	1926	1870
1.0%	844	892	1580	1624	1710	1692	1630
Taka diastase, 0.5%	82	125	219	1250	1778	2246	2222
1.0%	97	225	479	1707	1950	2030	2001
Pineapple juice (bromelin), 0.25 c.c.	1254	1419	1414	1426	1436	1436	1436
0.50 c.c.	713	1323	1322	1346	1346	1334	1346

While the results are not as consistent in every case as could be desired, a scrutiny of the data presented reveals increased protein solubility over the control with each enzyme treatment in the first hours of dispersion, except the one with taka diastase. The more substantial treatments caused speedy dispersal, but the concentration of protein tended to decrease toward the close of the period, probably owing to the removal of larger quantities of protein in the earlier stages of the reaction, as compared with gluten washed from doughs containing smaller enzyme concentration. This loss of protein would decrease the final concentration when compared with dispersions in which smaller amounts of protein had been initially removed.

No decreased solubility trend with small dosages of papain are apparent, while larger doses increase the ease of dispersal, doubtless

owing to a breaking down of the gluten complex. The activating effect of glutathione upon the native flour proteases is clearly demonstrated by the addition of graduated doses of yeast water, this effect increasing with an increase in quantity of glutathione. Pepsin did not show as high proteolytic activity as papain or pancreatin. The latter enzyme was the most active of any investigated with the possible exception of bromelin, and the determination of dispersion was stopped after 8 hours due to the leveling off of the rate of dispersion. Malt diastase and taka diastase showed unmistakable proteolytic activity, especially the former, but of a much lower order than the other enzymes in this study. This finding is in accord with the results presented by Read and Haas (1937), who proved the proteolytic effect of these enzyme preparations. The proteolytic effect of pineapple juice upon flour gluten was strikingly brought out, although the apparently larger effect of the smaller concentration as compared with the heavier dose, needs further study. The data relative to the degrading effect of

TABLE II

EFFECT OF VARYING CONCENTRATIONS OF PROTEOLYTIC ENZYMES UPON THE QUANTITY OF WHEAT GLUTEN WASHED FROM DOUGHS AND DISPERSED IN SODIUM SALICYLATE

Results Expressed as Percentages of Untreated Control

Treatment	Time of dispersion (hours)						
	1	2	4	8	12	24	36
	%	%	%	%	%	%	%
Papain, 0.002%	86	111	93	82	117	153	151
0.004%	182	149	141	280	256	—	140
0.008%	120	93	91	169	250	171	167
0.012%	118	197	441	425	315	153	146
0.020%	392	838	570	312	229	113	107
Yeast water, 10 c.c.	118	161	224	274	228	124	117
20 c.c.	174	351	351	306	229	111	—
30 c.c.	396	574	368	284	205	99	—
Pepsin, 0.1%	94	111	91	112	123	123	—
0.4%	594	709	495	288	211	103	—
Pancreatin, 0.01%	210	510	496	320	—	—	—
0.03%	1109	799	518	262	—	—	—
Malt diastase, 0.5%	111	205	526	273	239	133	123
1.0%	715	587	—	321	244	116	107
Taka diastase, 0.5%	70	82	85	—	253	155	146
1.0%	82	148	186	337	278	140	131
Pineapple juice, 0.25 c.c.	1063	934	550	282	205	99	94
0.50 c.c.	604	870	514	266	192	92	88

pineapple juice upon the flour gluten are also in line with the work of Read and Haas.

In Table II the solubility results are shown in terms of percentage of the control, untreated dough. These data present quite clearly the striking effects of enzymic treatments on gluten solubility. This is especially true in the instances of pancreatin and bromelin. The concentration of enzyme in the dough is reflected in the rate at which the gluten is dispersed in sodium salicylate; that is, increasing the enzyme concentration speeds up the rate of dispersion. The same relation is evidently true in the case of the activation of the flour proteases, as represented by the data for yeast water. Taka diastase has the smallest effect of any of the enzymes used in this investigation,

TABLE III

EFFECT OF INCREMENTS OF POTASSIUM BROMATE UPON THE DISPERSION OF WHEAT GLUTEN WASHED FROM DOUGHS CONTAINING DIFFERENT PROTEOCLASTIC ENZYMES

Results in Milligrams of Protein per 100 c.c. Dispersion

Treatment	Time of dispersion (hours)						
	1	2	4	8	12	24	36
Papain, 0.002%	952	1430	1513	1493	——	——	——
" " $KBrO_3$ 0.004%	670	1365	1545	1550	——	——	——
" " $KBrO_3$ 0.008%	464	1254	1478	1499	——	——	——
" " $KBrO_3$ 0.016%	353	1151	1499	1539	——	——	——
Papain, 0.001%	108	160	256	1174	1892	2280	2314
" " $KBrO_3$ 0.004%	111	154	268	1414	2138	2326	2326
" " $KBrO_3$ 0.008%	105	157	256	1112	1944	2292	2302
" " $KBrO_3$ 0.016%	103	151	254	1084	1926	2308	2320
Yeast water, 25 c.c.	117	311	624	1160	1430	1840	2006
" " " $KBrO_3$ 0.004%	103	282	601	1137	1384	1950	2018
" " " $KBrO_3$ 0.008%	122	305	621	1191	1402	1936	1910
" " " $KBrO_3$ 0.016%	114	236	433	940	1112	1812	2240
Pepsin, 0.4%	701	1077	1273	1458	1482	1494	——
" " $KBrO_3$ 0.016%	707	1154	1319	1385	1413	1430	——
Pancreatin, 0.03%	1308	1214	1331	1328	——	——	——
" " $KBrO_3$ 0.004%	1593	1499	1624	1610	——	——	——
" " $KBrO_3$ 0.016%	1171	1066	1245	1220	——	——	——
Malt diastase, 0.5%	114	180	656	1516	1920	1972	1978
" " " $KBrO_3$ 0.004%	91	151	684	1504	1960	2040	2040
" " " $KBrO_3$ 0.008%	97	148	419	1368	1950	2046	2046
" " " $KBrO_3$ 0.016%	86	154	336	1300	1904	2052	2080
Malt diastase, 1.0%	844	892	1580	1624	1710	1692	1630
" " " $KBrO_3$ 0.004%	331	741	1237	1545	1602	1516	1664
" " " $KBrO_3$ 0.016%	259	644	1197	1568	1652	1590	1636

TABLE III—*Continued*

Treatment	Time of dispersion (hours)						
	1	2	4	8	12	24	36
Taka diastase, 0.5%	83	145	336	1306	1916	2154	2154
" " " $KBrO_3$ 0.004%	88	160	430	1570	2040	2098	2074
" " " $KBrO_3$ 0.008%	83	160	382	1442	1978	2080	2052
" " " $KBrO_3$ 0.016%	80	151	259	980	1830	2218	2224
Taka diastase, 1.0%	160	599	1169	1744	1824	1688	1766
" " " $KBrO_3$ 0.004%	177	678	1380	1698	1722	1688	1744
" " " $KBrO_3$ 0.008%	105	468	1265	1732	1710	1710	1790
" " " $KBrO_3$ 0.016%	103	274	1020	1836	(1676)?	1790	1892
Pineapple juice (bromelin), 0.25 c.c.	1254	1419	1414	1426	1436	1436	1436
" " $KBrO_3$ 0.004%	183	422	1208	1778	1766	1802	1824
" " $KBrO_3$ 0.008%	103	194	838	1630	1922	2132	2178
" " $KBrO_3$ 0.016%	108	182	895	1688	2052	2188	2234
Pineapple juice (bromelin), 0.5 c.c.	713	1323	1322	1346	1346	1334	1346
" " $KBrO_3$ 0.004%	445	946	1459	1676	1664	1664	1698
" " $KBrO_3$ 0.008%	199	598	912	1734	1722	1722	1756
" " $KBrO_3$ 0.016%	143	547	895	1756	1778	1778	1824
Papain, 0.02%	137	553	1225	1800	1850	1834	——
" " Lemon juice (ascorbic acid) 0.5 c.c.	111	279	1054	1858	1920	1892	——
" " 1.0 c.c.	114	353	1072	1830	1880	1864	——
" " 2.0 c.c.	114	179	572	1528	1886	1880	——

and appears to depress the solubility. This depression of solubility becomes less evident as the concentration of enzyme is increased. This effect may possibly be explained by a coagulating action of lower concentration of the enzyme upon the flour gluten and agrees with the theory that the initial action of proteolytic enzymes upon gluten is of a coagulating rather than dispersive nature. The glutens washed from doughs treated with a higher concentration of enzyme go into solution much faster at first than the glutens from doughs treated with lower enzyme concentration, or the control-dough glutens, but the rate decreases and falls below the others as the dispersion progresses. The results presented in this table give the same picture as was shown in Table I, but in a more striking way.

In Table III the results of superimposing increments of potassium bromate upon enzyme-treated doughs are shown in milligrams of protein dispersed per 100 c.c. of solution. All the proteolytic enzymes which had been used in this study for determining the effects of enzymic action upon gluten solubility were examined in respect to their reaction

to the oxidizing effects of bromate. Three concentrations of the chemical were employed, the milder treatments corresponding roughly to commercial usage, while the higher doses would be equal to a fairly

TABLE IV

EFFECT OF INCREMENTS OF POTASSIUM BROMATE UPON THE DISPERSION OF WHEAT GLUTEN WASHED FROM DOUGHS CONTAINING DIFFERENT PROTEOCLASTIC ENZYMES

Results Expressed as Percentages of Unbromated Control

Treatment	Time of dispersion (hours)						
	1	2	4	8	12	24	36
Papain, 0.002% $KBrO_3$ 0.004%	70	95	102	104	—	—	—
" " $KBrO_3$ 0.008%	48	88	98	100	—	—	—
" " $KBrO_3$ 0.016%	37	80	99	103	—	—	—
Papain, 0.01% $KBrO_3$ 0.004%	103	96	105	120	113	102	100
" " $KBrO_3$ 0.008%	97	98	100	95	103	100	99
" " $KBrO_3$ 0.016%	95	94	99	92	102	101	100
Yeast water, 25 c.c. $KBrO_3$ 0.004%	88	91	96	98	97	106	101
" " " $KBrO_3$ 0.008%	104	98	99	103	98	105	95
" " " $KBrO_3$ 0.016%	97	76	69	81	78	98	112
Pepsin, 0.4% $KBrO_3$ 0.008%	180	122	103	91	90	88	—
" " $KBrO_3$ 0.016%	101	107	104	95	95	96	—
Pancreatin, 0.03% $KBrO_3$ 0.004%	122	123	122	121	—	—	—
" " $KBrO_3$ 0.016%	89	88	93	92	—	—	—
Malt diastase, 0.5% $KBrO_3$ 0.004%	89	84	104	99	102	103	103
" " " $KBrO_3$ 0.008%	85	82	64	90	102	104	103
" " " $KBrO_3$ 0.016%	75	86	51	86	99	104	105
Malt diastase, 1.0% $KBrO_3$ 0.004%	39	83	—	95	94	90	102
" " " $KBrO_3$ 0.016%	31	72	—	96	97	94	100
Taka diastase, 0.5% $KBrO_3$ 0.004%	106	110	128	120	106	97	96
" " " $KBrO_3$ 0.008%	100	110	114	110	103	97	95
" " " $KBrO_3$ 0.016%	96	104	77	75	95	103	103
Take diastase, 1.0% $KBrO_3$ 0.004%	111	113	118	97	94	100	99
" " " $KBrO_3$ 0.008%	66	78	108	99	94	101	101
" " " $KBrO_3$ 0.016%	64	46	87	105	92	106	107
Pineapple juice, 0.25 c.c. $KBrO_3$ 0.004%	15	30	85	125	123	126	127
" " " $KBrO_3$ 0.008%	8	14	59	114	134	148	152
" " " $KBrO_3$ 0.016%	9	13	63	118	143	152	156
Pineapple juice, 0.50 c.c. $KBrO_3$ 0.004%	62	71	110	124	124	125	126
" " " $KBrO_3$ 0.008%	28	45	69	129	128	129	130
" " " $KBrO_3$ 0.016%	20	41	68	130	132	133	135
Papain, 0.02% Lemon juice 0.5 c.c.	81	50	86	103	104	103	—
" " " " 1.0 c.c.	83	64	87	102	102	102	—
" " " " 2.0 c.c.	83	32	47	85	102	102	—

stiff treatment which in actual baking practice would tend to ruin the loaf of bread produced. Several enzyme concentrations were also employed.

Repression is clearly indicated in the lighter dosages of papain with all concentrations of bromate used, this effect increasing as the dose of enzyme was stepped up. At an increased concentration of papain no repression was evident for any bromate treatment. It

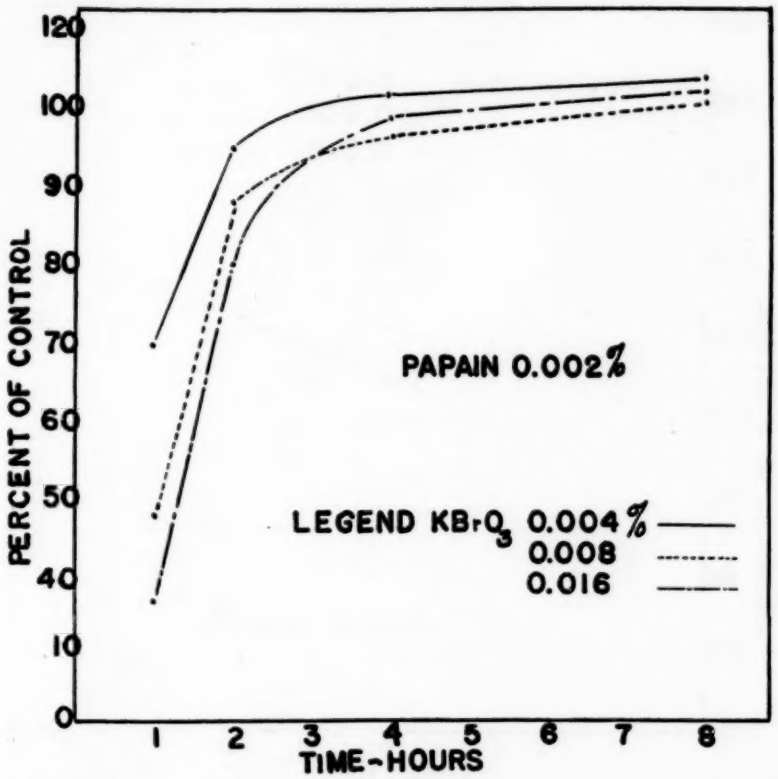

Fig. 1. Effect of increments of $KBrO_3$ upon the proteolytic activity of papain present in relatively low concentrations. Values refer to soluble protein as percent of the nonbromated control.

would seem that further work is needed regarding the repressive action of potassium bromate upon this enzyme. The effect of the bromate upon flour proteases activated by glutathione is not very clear, but repression appeared to take place at the highest concentration of bromate used, not at either of the two lower treatments. Further work is also needed here, with a comparison between activated flour proteases and doughs to which no activator or enzyme has been added. No repression was evident in the instance of pepsin and probably not

for pancreatin, but it was shown in the case of malt diastase, especially when 1.0% of enzyme was added to the dough mix. Taka diastase was also repressed.

Bromelin showed very distinct repression and appeared to be more sensitive to the effect of bromate than any other enzyme investigated in this study. Repression of papain activity by lemon juice was also shown, and may explain the favorable effect of this ingredient in

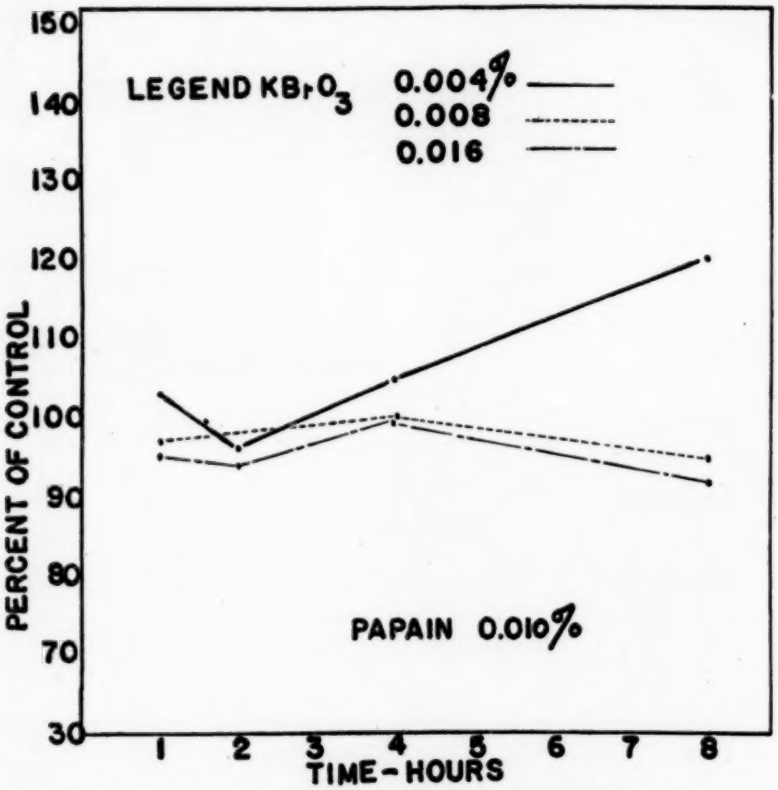

Fig. 2. Effect of increments of $KBrO_3$ upon the proteolytic activity of papain present in substantial amounts. Values refer to soluble protein as percent of the nonbromated control.

doughs, as noted by Melville and Shattock (1938), by its repressive action upon proteolytic enzymes.

In Table IV the results obtained with the use of bromate are shown as percentages of the control, the unbromated dough. In this table the data show repression of the activity of papain even with a concentration of 0.1% of the enzyme. Repression is also evident in the case of the activated flour proteases. No repression is shown for pepsin, at least at the commencement of dispersion. Bromate appears

to have an activating effect upon pancreatin when present in small dosages, but does seem to repress when present in heavier concentrations in relation to enzyme. The activity of malt and taka diastase are both influenced by bromate, the former to a much larger extent

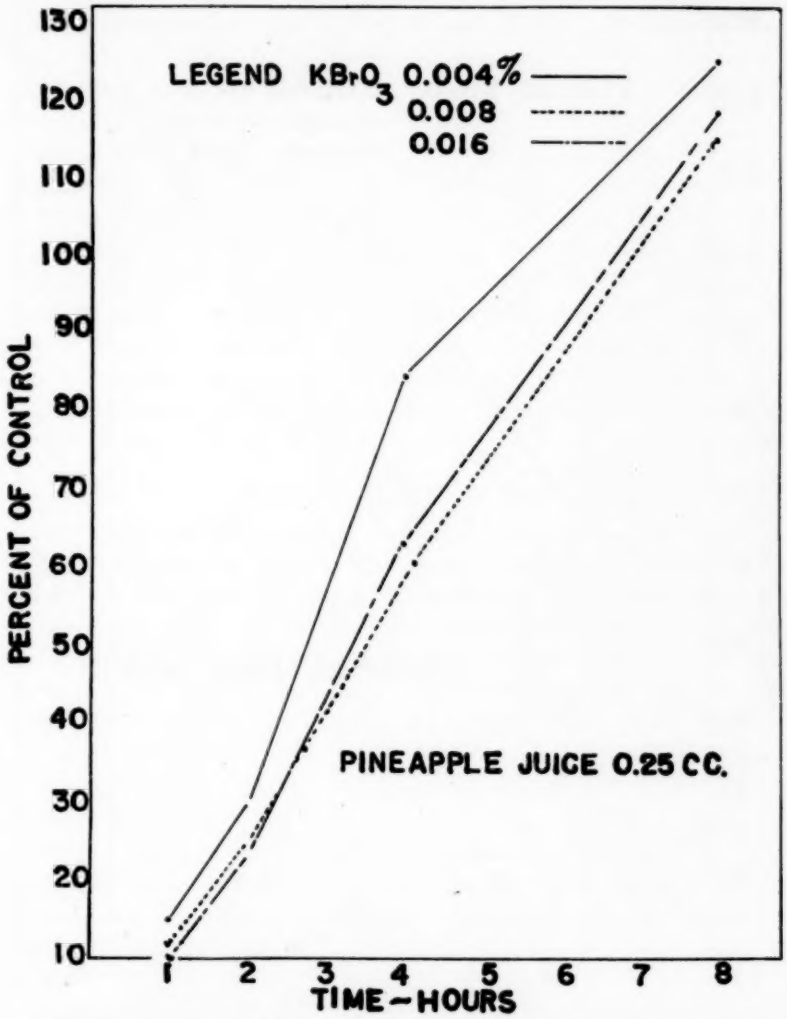

Fig. 3. Effect of increments of $KBrO_3$ upon the proteolytic activity of bromelin present in moderate concentrations. Values refer to soluble protein as percent of the nonbromated control.

than the latter. Small concentrations of bromate appear either to activate taka diastase or to inhibit an initial coagulative action upon the flour gluten. This point also needs further investigation. Bromelin is very strongly repressed by potassium bromate. The higher

percentages of protein apparently present in many of these dispersions after a number of hours is likely due to a lower rate of gluten disintegration, as compared with the control, at the earlier stages of dispersal, with consequently less protein removed from the system.

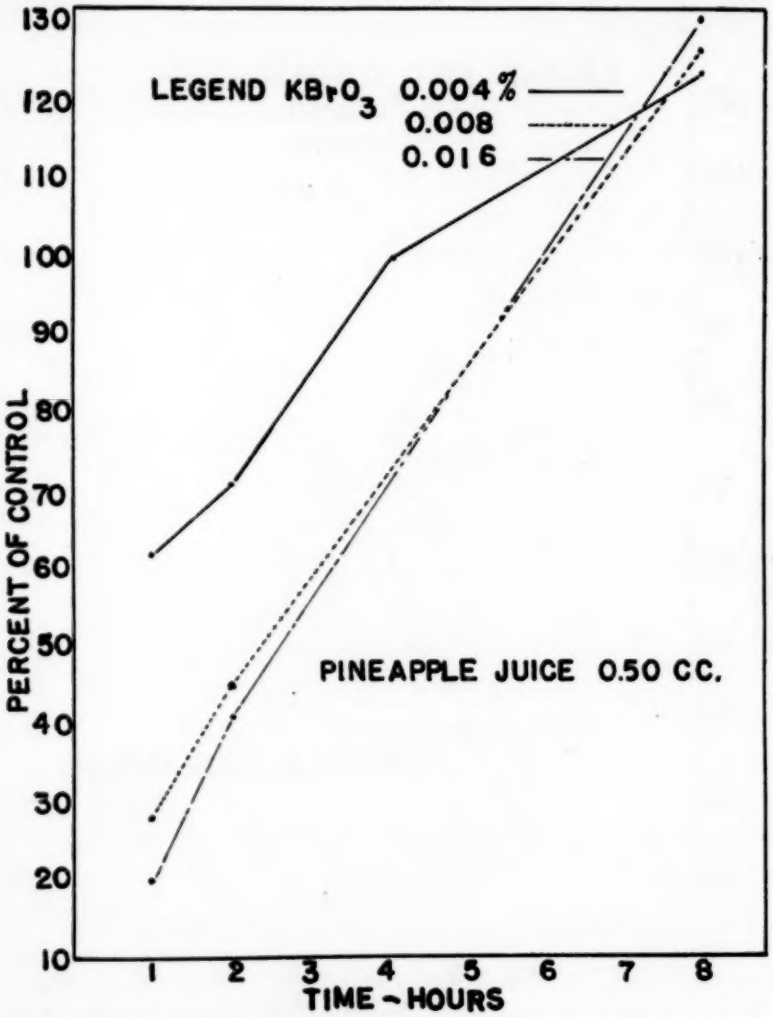

Fig. 4. Effect of increments of $KBrO_3$ upon the proteolytic activity of bromelin present in relatively high concentrations. Values refer to soluble protein as percent of the nonbromated control.

It appears probable that potassium bromate, when present in low concentrations in relation to enzyme, may exert an activating action upon proteoclastic enzymes, especially those of animal origin. A similar possibility was pointed out by Falk and Winslow (1918) when

digesting casein by trypsin or pancreatin. This effect is lost, and repression of enzymic activity sets in as the concentration of bromate is increased. In the instance of taka diastase, however, the situation may be somewhat different, as this enzyme appears to depress the initial solubility and the effect of bromate in increasing the solubility may be explained by inactivation of the enzyme at lower bromate concentrations. The results presented in this study are of a more or less tentative basis, and more intensive work is necessary to throw more light on the action of potassium bromate upon this class of enzyme.

Graphs (Figs. 1, 2, 3 and 4) show the repressive action of potassium bromate upon papain and bromelin, respectively. Two concentrations of enzyme and three of the chemical are presented in the instance of each enzyme. Striking differences between the sets of curves obtained for each enzyme are shown, and represent fundamental differences in their properties. In the case of papain the lower concentration of bromate to papain appeared to alter the three curves materially and showed little repression. In the instance of bromelin, little change in the general character of the curve is evident with change in the bromelin concentration.

Summary and Conclusions

A sample of hard red spring wheat flour was mixed into a dough in the usual manner for bread baking, and various concentrations of different proteolytic enzymes were added to the dough mix. Immediately following the mixing the gluten was washed from these doughs and dispersed in 10% sodium salicylate solution. The rate at which these doughs became dispersed in the solution was then determined by drawing aliquots at suitable intervals and determining the nitrogen content of the centrifuged liquid. The effect of increments of potassium bromate upon proteolytic activity was similarly investigated.

In the majority of instances the inclusion of an enzyme materially hastened dispersion of the gluten and this effect was found to increase with increasing enzyme concentration. Difficulty was also experienced in many instances in properly washing the glutens following enzyme treatment.

Of the enzymes studied, bromelin and pancreatin appeared to exert the greatest influence upon gluten dispersion, while taka diastase appeared to decrease the solubility. This effect of taka diastase may be explained by an initial coagulation of the gluten protein. Yeast water, which possessed no proteolytic activity itself, showed a decided activating effect upon the flour proteases. Malt diastase had a very noticeable influence upon gluten solubility, and is distinctly proteolytic in action.

Potassium bromate inhibited papain when present in relatively large concentrations in respect to enzyme. This effect was not very apparent when the enzyme concentration was increased. Bromelin was very strongly repressed at all concentrations used. Some repression of the activated flour proteases appeared to take place in the yeast-water treatment. Pepsin appeared to be the only enzyme investigated which did not evidence repression by bromate, but showed instead possible activation, especially with low concentration of the chemical. Several of the enzymes gave indications of possible activations by bromate when the chemical was present in relatively small quantities, whereas heavier dosages induced repression. An alternative explanation may be advanced in the case of taka diastase, where this effect of bromate may be attributed to repression of a possible coagulative effect of this enzyme upon the flour gluten. Further investigation is needed to elucidate these phenomena.

Acknowledgments

The writer wishes to acknowledge the aid of T. Sanderson, who mixed the large number of doughs required in this investigation. Grateful acknowledgment is also made of technical assistance from the WPA and NYA funds.

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THE COLLOIDAL BEHAVIOR OF FLOUR DOUGHS. IV. THE CAUSES OF THE INCREASE IN MOBILITY OF DOUGHS UPON PROLONGED MIXING[1]

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(Received for publication April 2, 1938)

In the normal course of mixing a dough there is an initial decrease in mobility which passes through a minimum and then begins to increase. The rate of this increase in mobility early attracted the interest of cereal technicians. A tendency for certain flours to increase more rapidly in mobility than others was early noted. Brabender (1932) considered this increase in mobility to be "a measure of the stability of the gluten against mechanical and chemical influences."

Brabender (1932a) demonstrated that the addition of a proteolytic enzyme to a dough would cause a much more rapid increase in mobility than in a normal dough. Katz (1934) found a great similarity between the increase in mobility of doughs made from flours that had been milled from slightly germinated wheat and the increase in mobility of doughs to which papain had been added. Landis (1935) developed a method for determining the proteolytic power of malts and similar preparations by use of the increase in mobility of the dough after addition of the preparation.

Rupp and Bailey (1937) made an extended study of the Landis method for determining proteolytic activity, and found it was only suitable for use with preparations of proteases that were free of diastases. Munz and Bailey (1937) investigating further into the effects of enzymes upon dough mobilities found that the effect of wheat malt in increasing mobility was due almost entirely to the α-amylase rather than to proteases. This finding was rather surprising because it brought a long neglected factor into the picture of the colloidal properties of doughs, namely, the role of the starch as something more than a mere filler and a source of sugar. Probably the reason for the neglect of the starch and the emphasis upon the protein lies in the early work employing dilute suspensions in viscosimeters. In such viscosimetric techniques the starch is almost unmeasurable in any effect upon the readings while protein seems all important, but in systems as low in water as doughs it is possible that the starch may be of considerable importance.

[1] Paper No. 1601, Journal Series, Minnesota Agricultural Experiment Station.

In order to test this hypothesis the investigation reported in the first section of this study (Markley, 1937) was instituted. As a result of this work it is apparent that starch, even in the absence of α-amylase. contributes to the increase in mobility during mixing. The starch-water system was at its minimum mobility during the first minute of mixing; after that the mobility increased in a decelerating manner. The rate of decrease in mobility of the starch-water dough was fully as rapid as that of many flour doughs. It is entirely conceivable that in some flours the only force active in increasing the mobility upon prolonged mixing is that inherent in the starch. The effect of the α-amylase now becomes logical; α-amylase activity is essentially that of liquefying starch, which would result in an increase in mobility of the system.

Bohn and Bailey (1936), using a modified Schofield and Scott-Blair technique for determining the elastic properties of doughs, found that overmixing in the farinograph reduced the elasticity of the dough, even though the mobility was not greatly changed. Since the tensile elasticity of a dough is primarily due to the gluten, it would appear that overmixing a dough past the point of minimum mobility does effect a measure of mechanical damage to the gluten structure.

In addition to the mechanical and enzymic damage to the dough structure upon prolonged mixing, there is also the chemical effect entering the picture. Munz (1935) has shown that cysteine accelerates the rate of increase in mobility while bromate retards it. This brings the major problem of oxidation-reduction potentials into consideration. He also found certain hydrogen-ion effects upon the rate of increase in mobility. Jørgensen (1936) found that glutathione would very greatly accelerate the increase in mobility during mixing. He considers glutathione to be an activator of latent proteases. Similar effects were observed when he used yeast extract.

Sullivan, Howe, and Schmalz (1936) confirmed Jørgensen's findings as to the destructive effect of glutathione upon doughs. Sullivan, Near, and Foley (1936, 1936a) found that the addition of wheat germ caused rapid increase in the mobility of doughs as measured in the farinograph. Some of the fatty constituents altered the shape of the curves, but the deleterious effects of the germ were mainly in the ether-insoluble fraction. The harmful constituents were found to be in the water-soluble portion of the germ, but are not as yet identified. McKillican (1936) has shown that the additions of such ions as sodium, chloride, calcium, magnesium, sulfate or carbonate in small quantities will reduce the rate of mobility increase in the farinograph.

Even the milling process is not without effect upon the rate of breakdown of the dough structure since Brabender and Abdon (1934)

have shown that the European type of wheat conditioners is able to influence appreciably the shape of the farinogram.

The rate of increase in mobility of a dough containing milk solids in the proportion of 6% of the flour weight after passing the minimum has been found by Skovholt and Bailey (1932) to be a practical measure of the adaptability of milks for use in bread baking.

It is not surprising after realizing the great number of factors involved that the rate at which the mobility of the dough increases has been very difficult to interpret. If only a single factor is involved, such as the testing of α-amylase for starch liquefying power or starch resistance to α-amylase, or determining the action of non-amylolytic protease preparations, or the testing of milks, the rate of increase in mobility of a dough is quite significant; but to attempt to use it in the differentiation of flours is not feasible in the light of present knowledge.

Summary

The rate of increase in mobility of flour doughs upon overmixing has been shown by different workers to be a function of many factors including mechanical degradation of the gluten structure, proteolytic activity, the thixotropic nature of the starch in the presence of water, the action of α-amylase, the presence of embryo constituents such as cysteine, glutathione and possibly certain unidentified substances, the ions in the dough solution, baking ingredients such as salt, bromate and milk, and even the manner in which the wheat was conditioned for milling.

The use of this rate of increase in mobility upon prolonged mixing is impractical as a simple and direct measure of flour strength, since it is the resultant of so many variable factors.

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A NEW ELECTRODIALYZER FOR THE PREPARATION OF BETA-AMYLOSE

SUTTON REDFERN

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(Read at the Annual Meeting, May 1938)

Taylor and Iddles (1926) have shown that it is very easy to separate alpha- and beta-amyloses by a combination of electrodialysis and electrophoresis. Since beta-amylose has great interest as a substrate for amylase action, suitable apparatus for its preparation is important. For this separation a new apparatus has been designed which makes use of Pyrex pipe. This pipe is standard for industrial purposes but has not, so far as I am aware, been used in the laboratory.

This pipe is made in four diameters from 1″ to 3″ and in lengths from 6″ to 120″. The ends of the pipe are flanged and can be securely clamped together with gaskets and metal flanges. By using different sizes, dialyzers can be made having a capacity as small as 78 milliliters and as large as desired. The one described herein and shown in Figure 1 holds 2 liters and is made from 3″ diameter pipe.

Details of the dialyzer are shown diagrammatically in Figure 2. Six-inch lengths of straight pipe are used for the end cells and an 18″ length for the middle cell. The end cells are clamped to the middle cell with the special metal flanges supplied for this pipe, with asbestos inserts to protect the pipe. Several kinds of gasket material are available, but the paraffined paper is the cheapest and very satisfactory. Two gaskets are used for each end and the parchment membrane is sandwiched between them. A double thickness of parchment paper is used for the lower membrane. Only moderate pressure on the flanges is needed for a water-tight joint.

The electrodes are cut from commercial cylindrical graphite electrodes. Each electrode is 1″ thick and is cut from a 2½″ diameter stock electrode. A hole is drilled in the center of the electrode and tapped with a ⅜″–16 tap. A ⅜″ carbon rod is threaded and screwed into this hole. A die cannot be used for threading this rod; it must be cut on a lathe. This graphite rod is long enough to extend out through the rubber stopper and serves both as a support for the electrode and as a means of connection to the source of current. To strengthen the rod a closely fitting Pyrex glass tube is sealed to it with DeKotinsky cement. The rubber stoppers are fitted as shown in Figure 2 with

connections for the circulation of water in the end cells. The water exit tube in the lower cell is placed horizontally under and touching the lower membrane. With this arrangement any trapped air or gas produced during the dialysis is easily removed through the three holes blown in the top of the tube. Otherwise the gas would gradually accumulate and insulate the electrode from the membrane. The bottom stopper is held in place with a six-inch circular piece of $\frac{1}{8}''$ sheet iron which is bolted to a metal flange. Holes are cut in this plate to slip over the glass tubing and electrode in the stopper.

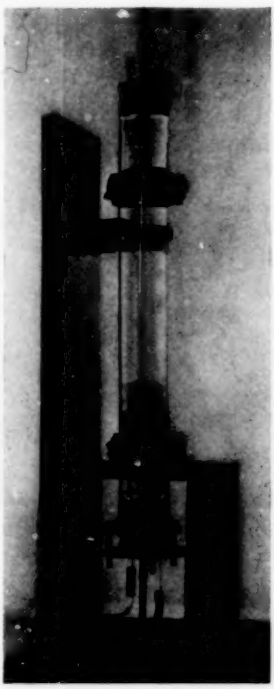

Fig. 1. Vertical electrodialyzer.

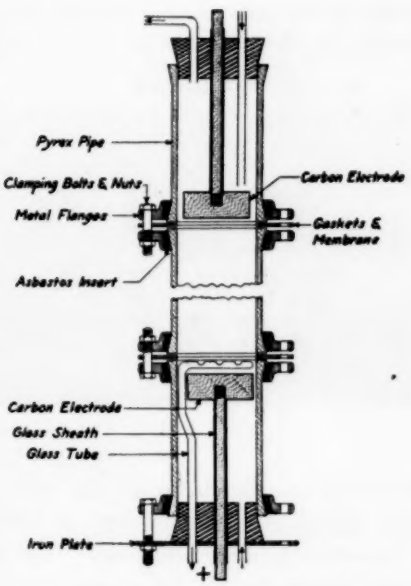

Fig. 2. Details of electrodialyzer.

A horizontal electrodialyzer can also be constructed with the pipe as shown in Figure 3. In this case the middle cell consists of a "T" fitting. This cell holds approximately one liter.

Preparation of Beta-Amylose

This apparatus has been used to prepare corn and potato beta-amyloses from the respective ground starches. The corn starch is ground for 168 hours in a ball mill and the potato starch 660 hours. A solution of the ground starch is prepared by adding gradually 70

grams of starch to about 600 milliliters of cold water while stirring with a high-speed mixer. Without a high-speed mixer the starch swells and forms lumps. The thoroughly mixed starch suspension is then added slowly to sufficient boiling water to make a final volume of two liters, and boiled several minutes. The boiled starch solution is cooled to about 30° C. and placed in the electrodialyzer. If the starch solution is cooled below 30° C. part of it irreversibly retrogrades and the final yield of beta-amylose is lowered. A little practice is necessary in order to put on the top cell without trapping air bubbles under the membrane, but the knack of doing this is easily acquired.

A potential of 750 volts is connected to the dialyzer, making the lower electrode the anode. A conventional voltage-doubler vacuum-tube rectifier is used to supply the direct current. The initial current is usually about 50 milliamperes but this drops during the first day to about 2 milliamperes. The potential is then increased to

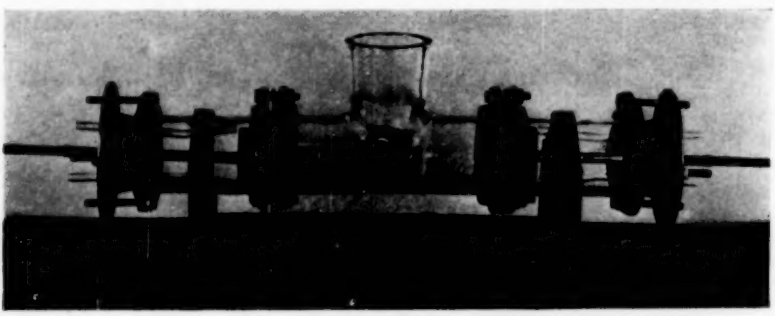

Fig. 3. Horizontal electrodialyzer.

1500 volts in order to make the separation as rapid as possible. The whole apparatus is placed in a 30° C. hotbox in order to prevent retrogradation of the amylose. The water in the end cells is changed several times daily. In about a week the starch solution separates into two distinct layers. The upper clear layer of beta-amylose, comprising about one-half of the cell, can then be siphoned off and is ready for use. Its concentration is usually around 2.5% and is determined by drying a 10 milliliter sample over night at 105° C.

A comparison was made of the action of malt amylase on the beta-amyloses from corn and potato starch by means of the rate curve method of Redfern and Johnston (1938). This method, in brief, consists of determining the rate of production of maltose by the same quantity of amylase acting on the same concentration of beta-amylose at the same temperature and pH. Figure 4 shows the results for the two amyloses. It is to be noted that the two curves are almost

superimposable, showing that these two amyloses, although from different starches, behave almost alike toward malt amylase. In a similar manner we expect to study the beta-amyloses prepared from

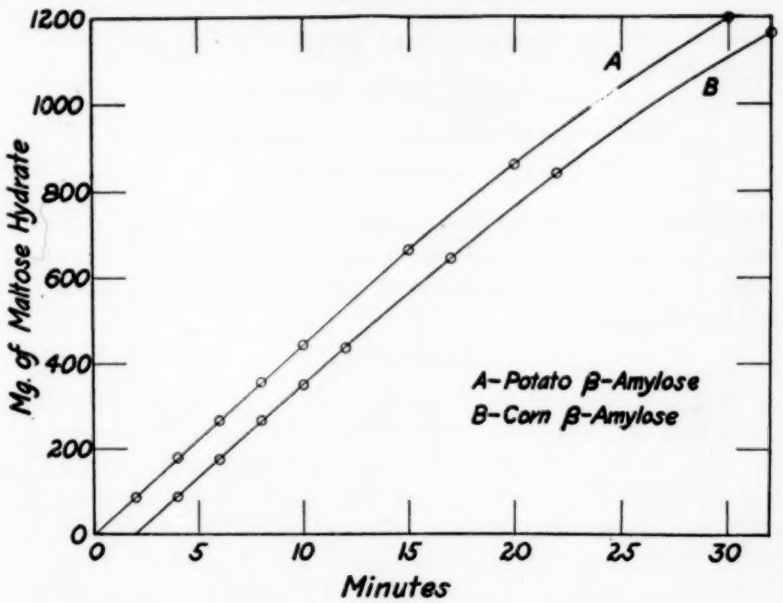

Fig. 4. Rate curves for β-amyloses.

other starches. This method will aid us in our study of the effect of the substrate on diastatic activity.

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LIPIDS OF WHEAT FLOUR

I. THE PETROLEUM ETHER EXTRACT

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(Received for publication May 28, 1938)

There are a few references in the literature to various common "constants" of wheat-flour fat but no work has been reported, so far as the authors are aware, on the kind and amounts of the fatty acids which are present.

Experimental

A freshly milled straight-grade hard spring wheat flour having an ash of 0.55% and a protein content of 16.65%, both calculated to dry basis, was chosen for analysis. The petroleum-ether extract was found to be 1.38% dry basis and the lipid (alcohol-ether extract) 1.81% dry basis. The flour was extracted with petroleum ether (b.p. 30°–60°), and the petroleum-ether extract was dried with anhydrous sodium sulfate and filtered. The filtrate was made up to volume and aliquots taken for various determinations. The methods used were those described in a previous paper (Sullivan and Bailey, 1936).

Physical and chemical characteristics of the petroleum-ether extracted material are recorded in Table I. The fat was saponified and, after removal of the unsaponifiable matter, the acids were recovered in the usual way.

The Saturated Fatty Acids

The saturated acids were separated by the Twitchell method, the percentage found being 15.6. The mixed saturated fatty acids were esterified and the methyl esters (6.5 g.) distilled at 2 mm. pressure from a Ladenburg flask. The following fractions were collected:

	Percent of total
Fraction 1 (145°–149°)	68.2
Fraction 2 (149°–153°)	14.7
Fraction 3 (residue)	17.1

Each fraction was saponified with 5% alcoholic potassium hydroxide and the acids recovered, washed, and dried. Each fraction was recrystallized from ethanol.

Fraction 1—0.1500 g. required 5.86 c.c. of 0.1N KOH = 256.0.
Fraction 2—0.1200 g. required 4.65 c.c. of 0.1N KOH = 258.0.

The neutralization equivalents from fractions 1 and 2 show both fractions to be palmitic acid whose molecular weight is 256.25. This was verified by melting points on both fractions. Mixed melting points with pure palmitic acid showed no depression with either fraction. Palmitic acid, therefore, made up approximately 83% of the saturated acid fraction.

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE PETROLEUM ETHER EXTRACT OF FLOUR

Specific gravity 26°/26°	0.9542
Refractive index 20°	1.4824
Acid value	21.6
Saponification value	177.8
Acetyl value	47.7
Reichert-Meissl value	7.9
Polenske number	1.1
Hehner number	87.0
Soluble acids as butyric	5.4
Ester number	156.2
Unsaponifiable matter (%)	5.48
Saturated acids (Twitchell) (%)	15.60
Unsaturated acids (by difference)	84.40
Iodine number of the total fatty acids	125.0
Thiocyanogen number of the total fatty acids	79.9
Iodine number of the unsaturated fatty acids	146.0
Mean molecular weight of total fatty acids	276.4
Mean molecular weight of saturated fatty acids	264.3
Mean molecular weight of unsaturated fatty acids	283.3

Fraction 3, after recrystallization, was still a light brown color, whereas the other two fractions were white even before recrystallization. Fraction 3 had a higher molecular weight but the amount was too small to identify with surety. It is planned to distill a larger sample of the mixed saturated methyl esters in order to see whether the remaining fraction is a mixture of stearic and lignoceric acid as in the wheat-germ saturated-acid fraction, or of different higher-boiling saturated acids.

The Unsaturated Acid Fraction

The total fatty acids recovered after removal of the unsaponifiable material had an iodine number of 125.0 and a thiocyanogen number of 79.9. The percent of the saturated acids was found to be 15.6. The iodine number of these saturated acids was less than 1. By using the formula as given by Kaufmann and Keller, it is possible to calculate the percentages of unsaturated acids. Substituting in the equations

given below, the following percentages are obtained: oleic acid 34.6%, linolic acid 46.0%, and linolenic acid 3.8%.

O = (100 − G) − 1.104 (iodine number − thiocyanogen number)
L = (100 − G) − 1.104 (2 thiocyanogen − iodine number)
Ln = − (100 − G) + 1.104 (thiocyanogen number)

(G is the percentage of the saturated acids; O, oleic acid; L, linolic acid; and Ln, linolenic acid.)

Bromination of the Unsaturated Fatty Acids

The unsaturated fatty acids (4.02 g.) were brominated in dry ether at − 10° C. After standing three hours the precipitate was filtered through a sintered glass crucible (10G3). The linolenic hexabromide recovered weighed 0.11 g. and melted at 177–9° C. The per cent of α linolenic acid found was therefore 1.00. The hexabromide dissolved completely in boiling benzene, indicating the absence of more highly unsaturated acids.

The filtrate from the small amount of hexabromide was evaporated *in vacuo* and taken up in petroleum ether (30–60°). Linolic tetrabromide (2.35 g.) was obtained. The filtrate from this precipitate was evaporated to approximately one-third its volume. An additional 0.24 g. of linolic tetrabromide was obtained, making a total of 2.60 g. linolic tetrabromide of 64.67%. The percentage of α linolic acid, therefore, is 30.22. The melting point of the linolic tetrabromide after recrystallization from ligroin was 114°–114½° C.

Anal. calculated for $C_{18}H_{32}O_2Br_4$: Br: 53.33
Found: Br: 53.90

The α linolenic found by bromination was 1.00% of the unsaturated acids or 0.84% of the total acids. The amount is probably somewhat low, owing to the small sample and the low content of linolenic acid. The α linolic acid, 30.22 g. as given above for the unsaturated acids, was 25.49% of the total acids. Using these figures, the percentage of β acids can be calculated from the total amount of the acids as found by the Kaufmann-Keller procedure. The percentages of the acids were as follows:

	%
Total saturated acids (83% palmitic acid)	15.6
Total unsaturated acids	84.4
α linolenic acid	0.84
β linolenic acid	2.96
α linolic acid	25.49
β linolic acid	20.51
Oleic acid (by difference)	34.60

Unsaponifiable Material

The unsaponifiable material from petroleum ether extract of flour was determined by the Kerr-Sorber method and found to be 5.49% and 5.47% on duplicate tests, or an average of 5.48%.

The percentage of free sterols was determined in the flour fat (2.828 g.) by adding 25 c.c. of 95% aldehyde-free ethanol and adding 25 c.c. of 90% ethanol containing 0.3 g. digitonin at 70° C. After standing 48 hours, the solution was filtered. The digitonin steride on drying weighed 0.1380 g. or 1.25% free sterols.

The percentage of mixed sterols in the unsaponifiable material was determined by taking 0.2973 g. of the unsaponifiable fraction and dissolving in 40 c.c. of 95% aldehyde-free ethanol. Digitonin, 0.5 g. in 50 c.c. 90% alcohol, was added at 70° C. After standing 24 hours, the solution was filtered through a 10G4 sintered glass crucible. The digitonin steride weighed 0.5088 g.; or 47.23% of the total unsaponifiable material was sterols. This fraction, therefore, contained 52.77% of material not precipitated by digitonin. The combined sterols in the flour fat can be calculated from the above figures and was found to be 1.34%. Probably most of the combined sterols of flour occur as sitosterol palmitate.

Discussion

The petroleum ether extract from flour differs from the wheat-germ lipids in having somewhat less unsaturation. Less linolic acid and more oleic acid was found. The percentage of total saturated acids is approximately the same as found for the wheat-germ lipids. The petroleum ether extract from wheat flour has a considerably higher Reichert-Meissl number than wheat-germ extract, indicating about ten times the amount of volatile soluble fatty acids.

All the flours examined had a somewhat higher content of unsaponifiable material than did the wheat germ. A significant difference was also noted in that, whereas about 70% of the unsaponifiable material from wheat germ is precipitated by digitonin, only about half of the unsaponifiable material from flour is precipitated by the same reagents. This test has been confirmed on several different flours.

This preliminary work on the flour lipids will be continued, especially as regards further investigation of the saturated acid fraction, the unsaponifiable material, and the volatile fatty acids. We are particularly interested in the different behaviour of the flour and germ lipids in baking.

Summary

A preliminary study of the fats extracted by petroleum ether from wheat flour indicates that wheat flour contains considerably more

volatile fatty acids than wheat germ. As is the case with the wheat-germ lipids, the bulk of the saturated fraction is palmitic acid. The same unsaturated acids, oleic, linolic, and small amount of linolenic acid, are present as in wheat germ. Wheat flour contains a somewhat higher amount of total unsaponifiable material than the germ with, however, only approximately half the unsaponifiable fraction being precipitated by digitonin.

Literature Cited

Sullivan, B., and Bailey, C. H.
1936 The lipids of the wheat embryo. I, The fatty acids. J. Am. Chem. Soc. **58**: 383–390. The lipids of the wheat embryo. II, The unsaponifiable fraction. J. Am. Chem. Soc. **58**: 390–393.

BOOK REVIEWS

The Soybean Industry. By A. A. Horvath. The Chemical Publishing Co. of N. Y., Inc., New York, N. Y. 190 pages. Price $4.00.

As might be expected from the title, the subject matter of this book is confined almost exclusively to the industrial utilization of soybeans. Possibly because the author has already written a most useful book entitled "Soybeans as a Human Food," we find only a bare mention of foods in this newer work.

The author is well qualified to speak and write on soybeans, either as food or as materials for industrial use, having had wide experience both in the land of the soybeans and in this country. Because much of the information found in this book is evidently old and because considerable attention is given to data obtained in Asia, where the author spent some time, one might be tempted to undervalue its importance. Information of this kind, which in all probability would otherwise have remained inaccessible to American readers, might well prove valuable to our workers on soybeans if for no other reason than that such knowledge and information have a broadening influence and provoke new ideas.

The book contains some twenty chapters, most of them covering less than ten pages each. The more important chapters are: Phosphatids, 29 pages; Industrial Protein, 25 pages; Technological Uses of Soybeans, 15 pages; and Plastics, 12 pages. Some of the chapters, while important, are highly theoretical, especially those on Solvent Extractions, Pressure Oil Milling, and Press Oil. The last-named describes the results of old experiments carried on under Asiatic conditions.

The chapter on Industrial Protein contains a mass of information regarding the reactions of the soybean proteins with various reagents, and much of this may prove very suggestive and helpful.

Throughout the book glaring errors, omissions, and inconsistencies may be noted, indicating that it must have been written hurriedly. This is unfortunate because the book does contain a great deal of information and these defects could easily have been prevented had the manuscript been written with greater care and been properly edited.

Such an expression as "to in the neighborhood of" instead of "to approximately" is rather awkward, to say the least. On one page is the word "centrifuging," on another "centrifugalization." Degrees in "F" and "C" are used indiscriminately. "Hydrophylic" on one page becomes "hydrophilic" on another. The statement, "The strength of lye shall be 20° for hydraulic oil," is closely followed by the statement that "Hydraulic oil shall be agitated at 20–24° C." The sentence beginning with ".1–25% lecithin" is evidently an error, but whether 0.1–2.5% or 0.1–0.25% is meant is for the expert to guess. By "light of low density" is probably meant "light of low intensity."

No mention is made regarding the danger of loss of phosphatides of soybeans, which contain over 12% moisture, when stored at too high a temperature. There

is little concerning the spoilage of oil due to rancidity and how such losses may be minimized.

The statement, "Visible light of 450–600 $\mu\mu$ causes neither ketone nor aldehyde formation" (quoted from the German), is likely to give an erroneous idea in view of the recent publications, in this country, on the photochemical effect of the various wave lengths of light on the spilage of oils. The further statement that "A faint yellow filter protects soybean oil from damage by visible light" is too indefinite to be of any real help.

It would have been helpful to the reader if in most instances the name of the patentee as well as the number of the patent had been given.

This is the latest though by no means the last word on the industrial uses of soybeans. Those who like to keep in touch with the broadening aspects of the subject will want to read this book. Though faulty in many small particulars, it still will be found to contain much of value and will no doubt prove an inspiration to many. The bibliography of 70 references includes many which are not readily available to the American reader and the inclusion of these citations is a help.

There is little excuse on the part of the publishers for inserting 13 pages of coarsely printed advertising material between the end of the text proper and the index, unless it were to make this relatively small book of about 45,000 words appear that much more voluminous. A book thus treated is most often cheapened, not in price but in the mind of the reader. To pay $4.00 for a book of this type is certainly not a bargain.

J. A. LeClerc

I Depositi Di Cereali. By Arnaldo Luraschi, President of the National Fascist Federation of Breadmakers and Allied Trades, Rome, Italy. Published and sold by the Federation, Piazza S. Sonino, 2, Rome. 678 pages, with tables, charts and illustrations.

Dr. Luraschi points out that in a program for national agricultural independence and self-sufficiency, the mass storage of cereals becomes a stabilizing factor of the most critical importance. Insurance against deterioration by insects, molds, etc., during storage, must be constantly maintained, and this is a responsibility of the cereal technologist.

Written in Italian, the book is devoted to an exhaustive, authoritative, and up-to-date consideration of all matters relating to causes of grain deterioration in storage, and to technological methods available for the prevention and control of deterioration. The author has assembled and organized a vast quantity of material heretofore available only in widely scattered journals and reports published in various languages.

A translation of the book into English would be heartily welcomed by all English-speaking cereal technologists.

M. J. Blish